THE AMERICAN JOURNAL OF PHYSIOLOGY

EDITED FOR

THE AMERICAN PHYSIOLOGICAL SOCIETY

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VOL. LIII—No. 2

Issued September 1, 1920

BALTIMORE, U. S. A.

1920

tered as second class matter, August 18, 1914, at the Post Office at Baltimore, Md., under the act of March 3, 1879. Acceptance for mailing at special rate of postage provided for in Section 1103, Act of October 3, 1917. Authorized on July 5, 1918

THE AMERICAN JOURNAL OF PHYSIOLOGY is issued monthly by the American Physiological Society under the direction of the Council of the Society. From two to three volumes each of about five hundred pages are published yearly. The subscription price per volume in the United States and Canada is $5.00; in other countries $5.25.

Manuscripts submitted for publication may be sent to any member of the Council or to the Managing Editor, Dr. D. R. Hooker, Johns Hopkins Medical School, Washington and Monument Streets, Baltimore, Md. They should be packed flat, carefully protected from injury and insured.

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THE AMERICAN
JOURNAL OF PHYSIOLOGY

VOL. 53 SEPTEMBER 1, 1920 No. 2

BLOOD REGENERATION FOLLOWING SIMPLE ANEMIA

I. Mixed Diet Reaction

G. H. WHIPPLE, C. W. HOOPER AND F. S. ROBSCHEIT

From the George Williams Hooper Foundation for Medical Research, University of California Medical School, San Francisco

Received for publication April 3, 1920

This series of papers deals with the regeneration of red cells and hemoglobin following simple anemia and the influence of diet factors upon this reconstruction. It will be shown that the curve of hemoglobin regeneration can be influenced at will by various diet factors. We believe that it is desirable to mention at least two lines of investigation which are being followed in this laboratory. To determine the value of various food factors when given alone or combined with other substances. To determine further the few or many substances which promote speedy regeneration of hemoglobin and red cells or to ascertain the optimum food combinations which will give a maximum blood regeneration following simple anemia. Inorganic substances and certain drugs are being investigated and this work will be presented in its proper place. Some experiments will deal with splenectomized dogs as well as bile fistula dogs but we prefer to present at a later time the bulk of our work on splenectomized animals which deals with the relation of splenectomy to blood regeneration under fixed experimental conditions. A preliminary report covering a part of this anemia work has been published elsewhere (1).

This work on blood pigment regeneration forms an essential part in any study of "pigment metabolism of the body." It is obviously closely related to a study of *bile pigment excretion* which was first taken up in our work and has been reported in part in earlier publications (2), (3). It will be recalled that the excretion of bile pigments may be

influenced or modified by various diet factors. For example, meat will cause increased flow of bile but a decided drop in total bile pigments. Carbohydrate, on the contrary, will reduce the flow of bile but increase the total bile pigment output. Decreased functional activity of the liver is associated with a decided fall in bile pigment elimination. We have assumed on the basis of much experimental evidence that the liver plays a *constructive* rôle in the bile pigment output. We hope to show the same relationship on the part of the liver to the *constructive mechanism* of blood regeneration.

We wish to emphasize that a curve of blood pigment regeneration cannot be established without accurate determination of at least two factors,—hemoglobin and blood volume. With a knowledge of these two factors we can estimate the total volume of hemoglobin pigment in the body circulation—the "pigment volume." Also with the hematocrit values we are able to compute the total volume of red cells in the body circulation. Reasonably accurate methods for the determination of circulatory blood volume are of recent development. A critical review of many factors in this blood volume work and comparison of various methods have been published from this laboratory very recently (4). The method used in this laboratory for accurate determinations of hemoglobin has been recently described by F. S. Robscheit (5).

Practically all these anemia blood regeneration experiments were performed upon dogs born and raised in our kennels—a bull dog cross which gives a very active, vigorous and healthy laboratory animal. Unless otherwise noted, the dogs were in fine normal condition during the entire experiment. These dogs will eat the food mixtures as a rule without any delay or wastage. They have been immunized against distemper and are kept in a separate room to obviate any cross infections by transient animals.

METHODS

The blood volume method used in these experiments has been described in detail elsewhere (4). In some of the earlier experiments dry oxalate was used for blood collection instead of isotonic fluid oxalate and in these experiments the calculated blood volume is too high. A note will be made in all such experiments as a correction cannot be introduced because the amount of solid oxalate and the corresponding shrinkage of cells was an unknown variable.

It may be stated in a word that the blood volume method consists in the introduction of a measured amount of a dye "brilliant vital red" into the blood stream. After a four minute period the dilution of the dye in the plasma is colorimetrically determined. "Brilliant vital red" has been furnished us through the courtesy of Dr. H. M. Evans of the Department of Anatomy and we wish to acknowledge many favors and valuable advice given us by Doctor Evans. The red cell hematocrit is read in an accurately calibrated centrifuge tube into which blood has been drawn, using an isotonic sodium oxalate solution. It is then a simple matter to calculate the plasma volume, red cell volume and total blood volume. This method can be quickly and accurately performed. It causes the dog a minimal degree of inconvenience, only that due to a hypodermic needle puncture of a vein, and the loss of only 35 cc. of whole blood. This is such a small amount of blood removed from the large circulating blood volume that we do not include it in our calculations and feel that no secondary anemia factors are added to complicate the reaction curve following the initial bleeding.

The hemoglobin determinations are made by means of a modification of Palmer's method, recently described in detail by one of us (5). This insures an accurate measure of the hemoglobin, as relatively large amounts of packed red cells are used. In some of the earlier experiments the Sahli hemoglobin tubes were used and in some instances these tubes had faded, giving hemoglobin values which were too high. The base line of any experiment although too high will not disturb the fairly accurate curve of regeneration which is more important. A footnote will be appended to all experiments in which the Sahli readings are given.

Red and white cell counts are made in the routine manner. Identical counts have been repeatedly obtained by venous puncture and from a freely bleeding ear puncture. The former is now our routine procedure.

The simple anemia is produced in the following manner: A simple blood volume is performed. The next day the dog is bled one-fourth the determined blood volume. This is easily done by inserting a needle into the jugular vein and aspirating the blood into a calibrated flask containing oxalate. The following day the same amount of blood is aspirated in exactly the same manner. During these two bleeding days and the next resting day the dogs are on a bread and milk diet. Following the resting day a second blood volume is done to determine the actual amount of anemia produced. The calculated and actual figures do not correspond but too many factors enter this equation to permit a

discussion at this time: for example, the reserve of blood cells thrown in from the marrow, to mention only one. At times a third bleeding is necessary if the reserve has been too great. A third blood volume is then done. The dog is then placed upon a fixed diet and complete blood volume, hemoglobin and blood cell determinations are done once each week thereafter. Special care is taken to insure a sufficient food ingestion based on the number of calories and nitrogen intake. The weight curve is a good index of the general nutrition.

The dogs are kept in individul cages which are comfortable and of suitable size to permit of much exercise. The cages are cleaned once daily by the attendant, but all feeding is done by the person in charge of the experiment. The mixed foods are usually eaten at once when placed each morning in the cage. Fluids are usually given by stomach tube. Water is furnished in the cage at all times except in metabolism experiments when it is usually given by stomach tube. It should be emphasized again that these dogs were raised in the laboratory and are therefore healthy, vigorous and very active at all times. The laboratory routine disturbs them not at all and the performance of the blood volume requires only a few minutes. They will eat all manner of food mixtures with relish and alacrity. Unless otherwise noted, these dogs are in their usual healthy, active condition throughout the entire experiment. "Mixed diet" in these experiments indicates a mixture of food materials obtained from the University Hospital, consisting of bones, bread, cooked meat, potato, rice, macaroni and general table scraps.

EXPERIMENTAL OBSERVATIONS

The dogs used in most of the experiments tabulated below are young animals in the active growth period,—one year of age or less. Such animals are increasing in size, weight and strength and the demand for tissue building or growth factors is acute. It is therefore of interest to keep this fact in mind during our analysis of the subsequent experiments. It might be assumed that the reconstruction of red cells in a rapidly growing animal might be handicapped by the tissue demand for normal growth factors. On the other hand, it may be argued that the growth capacity of the younger organisms might be greater as regards the construction of tissue cells, including the red cells. When we review the experiments to be submitted in subsequent communications it may be stated that the difference between the adult and the young dog is not great as regards the capacity of the animal to regener-

ate new red blood cells. This statement applies to young dogs between 6 and 12 months of age and takes into consideration the individual variations which are met with in different dogs. It is possible that this statement may not hold for pups less than 6 months of age. In general we may be safe in stating that if there is any difference between young and adult dogs, there is a slight difference in favor of the adult

TABLE 1

Blood regeneration—mixed diet. Dog 19–93. Bull pup, female, age 13 months

DATE, 1919	PIGMENT VOLUME = Hb. PER CENT TIMES BLOOD VOLUME	BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	R. B. C. HEMATOCRIT	Hb.	COLOR INDEX	R. B. C. MILLION	W. B. C.	WEIGHT	BLOOD PER KILOGRAM	REMARKS
		cc.	*cc.*	*cc.*	*per cent*	*per cent*				*kgm.*	*cc.*	
8/11	1780	1280	580	688	53.7	139	0.91	7,6	16,2	12.6	101	
8/12	Diet: Bread and milk											
8/12	Bled 320 cc. Slight distress. Injection 100 cc. N/1 salt solution											
8/13	Bled 320 cc. No distress											
8/15	614	986	732	249	25.3	62	0.97	3,2	12,8	13.0	76	Normal
8/15	Diet: Mixed diet											
8/21	658	1023	716	292	28.5	64	0.80	4,0	6,2	13.25	77	Diet poor in meat
8/28	962	1210	728	465	38.4	79	0.69	5,7	10,8	13.3	91	
9/4	1280	1308	724	572	43.7	98	0.82	6,0	10,0	14.1	93	More meat in food
9/11	1538	1420	673	733	51.6	108	0.64	8.4	8,8	14.3	99	
9/18	1612	1355	644	700	51.6	119	0.68	8,7	10,4	14.5	94	
9/25	1726	1444	681	748	51.8	120	0.69	8,7	12,6	15.5	93	
10/2	1830	1490	675	793	53.2	123	0.72	8,5	12,0	15.7	95	

dogs who at times seem to show a slightly shorter period of blood regeneration under similar circumstances.

The first three experiments (tables 1, 2 and 3) were performed upon three young dogs of the same litter, all of the same weight, activity and general appearance. It will be observed that there are individual differences even under these favorable conditions. It is possible that some of this variation may be explained by individual preferences of the

different dogs for various food factors. They were all given an excess of mixed food from which of course they could pick out the bits of food which they preferred. During much of this diet experiment the "mixed food" was poorer than usual in meat and bones. This explains the fact that the period of blood regeneration appears somewhat longer than in some of the subsequent experiments. The explanation for this fact is found in paper IV of this series, which shows the remarkable efficiency of a meat diet in promoting blood regeneration.

TABLE 1-B

Experimental history. Dog 19–93

EXPERIMENT	DIET	BLOOD REGENERATION		WEIGHT	REMARKS
		Pigment volume	Blood per kilogram		
				kgm.	
Begin 1/16/19	Rice, potato, milk	1435	126	10.95	Born July 11, 1918
Bled 590 cc.		594	86	10.25	
End 3/7/19		1098	113	10.35	Maximum regeneration 5 weeks
Begin 3/17/19	Rice, potato, milk (repeat)	1040	89	11.35	Table 42
Bled 502 cc.		566	82	11.15	
End 4/30/19		1313	107	11.15	Complete regeneration 4 weeks
Begin 8/11/19	Mixed diet	1780	101	12.6	Table 1
Bled 640 cc.		614	76	13.0	
End 10/2/19		1830	95	15.7	

It is to be noted that all these three dogs increased markedly in body weight. It should be stated that this was a general growth with increase in size, length of limb and body, not merely a deposit of fat. One is not surprised to note a gradual increase in blood and plasma volume during the experiment. The total volume of red cells, hematocrit reading of red cells and hemoglobin follow curves which are parallel. The color index is almost constantly between 0.65 and 0.85 and there is not a very strong tendency in these experiments for the color index to drop much below normal in the first two weeks following the hemorrhage. We shall not attempt at this time to discuss the fluctuations in white blood cells which are tabulated.

The "pigment volume" is a convenient and expressive term which indicates the total volume of circulating or effective blood pigment in the blood stream at the time of estimation of blood volume and hemoglobin. The *pigment volume is the product of blood volume times per cent hemoglobin.* In all tables the *pigment volume* is the first value

TABLE 2

Blood regeneration—mixed diet. Dog 19–94. Bull pup, female, age 13 months

DATE, 1919	PIGMENT VOLUME = Hb. PER CENT TIMES BLOOD VOLUME.	BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	R. B. C. HEMATOCRIT	Hb.	COLOR INDEX	R. B. C. MILLION	W. B. C.	WEIGHT	BLOOD PER KILOGRAM	REMARKS
		cc.	*cc.*	*cc.*	*per cent*	*per cen*				*kgm.*	*cc.*	
8/11	1705	1242	572	658	53.0	137	0.78	8,8	7,6	11.0	113	
8/11	Diet: Bread and milk											
8/12	Bled 310 cc.											
8/13	Bled 310 cc. No distress											
8/15	540	1000	761	230	23.0	54	0.84	3,2	6,4	13.5	74	Normal
8/15	Diet: Mixed diet											
8/21	686	1090	768	306	28.1	63	0.67	4,7	8,6	13.65	80	Diet poor in meat
8/28	800	1194	812	370	31.0	67	0.78	4,3	6,0	13.4	89	
9/4	972	1292	843	443	34.3	75	0.66	5,7	12,0	13.9	93	More meat in diet
9/11	1032	1328	836	489	36.8	78	0.67	5,8	8,2	14.0	95	
9/18	1062	1205	736	465	38.5	88	0.69	6,4	6,2	13.8	87	
9/25	1226	1295	760	524	40.4	95	0.68	7,0	7,8	14.45	90	
10/2	1298	1276	710	554	43.4	102	0.74	6,9	17,4	14.55	88	More meat in diet
10/9	1520	1463	827	624	42.6	104	0.73	7,1	13,6	15.5	95	
10/23	1550	1463	823	620	42.4	106	0.74	7,2	8,8	16.25	90	

given as we believe it gives the best general index of the curve of blood regeneration.

Table 2, dog 19-94 is an experiment with a dog presenting some unknown abnormal factor. This dog at times shows an eosinophilia yet only an occasional parasite egg can be demonstrated in the feces.

Treatment by oil of chenopodium and santonin has yielded no results. The dog is not as well nourished as the others of this litter and at times presents a slight relative degree of anemia. This fact is to be considered in a study of the abnormally slow blood regeneration in this dog. In spite of this the dog gained about eleven pounds during the course of the experiment.

Table 3 at the start of the experiment shows the remarkably high figure (152 per cent hemoglobin) which may be observed in normal dogs. Red blood counts of 7 to 9 millions are the rule.

TABLE 2-B

Experimental history. Dog 19–94

EXPERIMENT	DIET	BLOOD REGENERATION		WEIGHT	REMARKS
		Pigment volume	Blood per kilogram		
				kgm.	
Begin 1/16/19	Bread, 300	1250	126	10.65	Born July 11, 1918
Bled 620 cc.	grams, milk	462	84	10.00	
End 3/7/19	500 cc.	899	94	11.70	Maximum regeneration 5 weeks
Begin 3/17/19	Bread, 300	1146	93	13.05	Table 27
Bled 608 cc.	grams, milk	542	78	12.40	
End 4/30/19	500 cc. (repeat)	1031	99	13.20	Maximum regeneration 5 weeks
Begin 8/11/19	Mixed diet	1705	113	11.00	Table 2
Bled 620 cc.		540	74	13.5	
End 10/23/19		1550	90	16.25	

October 9. Two doses of oil of chenopodium, 48 hours apart. Few ova found. No worms expelled. Last dose followed by santonin mixture. No effect.

Table 4 shows an experiment upon a young dog (6 months) in which one week's diet of rice, potatoes and milk followed the bleeding—then the usual mixed diet. The dog did not eat the rice, potato and milk diet and lost much weight. Also she developed signs of mild distemper, but this soon cleared up after being put on a liberal mixed diet. There was then a rapid gain in weight as well as in blood regeneration. The leucocytosis is probably to be explained by the mild infection with distemper. The total regeneration of red cells is complete in one month.

TABLE 3

Blood regeneration—mixed diet. Dog 19–95. Bull pup, female, age 13 months

DATE, 1919	PIGMENT VOLUME = Hb. PER CENT TIMES BLOOD VOLUME	BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	R. B. C. HEMATOCRIT	Hb.	COLOR INDEX	R. B. C. MILLION	W. B. C.	WEIGHT	BLOOD PER KILOGRAM	REMARKS
		cc.	*cc.*	*cc.*	*per cent*	*per cent*				*kgm.*	*cc.*	
8/11	2088	1369	568	794	58.0	152	0.88	8,6	10,4	13.2	104	
8/11	Diet: Bread and milk											
8/12	Bled 342 cc.											
8/13	Bled 342 cc. No distress											
8/15	750	1005	700	300	29.8	74	1.03	3,6	8,4	13.65	74	Normal
8/15	Diet: Mixed diet											
8/21	843	1106	728	367	33.2	76	0.93	4,1	17,8	14.2	78	Diet poor in meat
8/28	829	1066	747	414	35.5	78	0.75	5,2	10,2	14.0	83	
9/4	954	1243	814	423	34.0	77	0.71	5,4	19,0	14.65	95	More meat in diet
9/11	1313	1338	726	600	44.8	98	0.65	7,5	13,2	14.5	92	
9/18	1325	1243	674	552	44.4	107	0.74	7,2	16,0	14.65	85	
9/25	1730	1412	678	720	51.0	122	0.74	8,2	13,0	15.25	93	
10/2	1874	1410	618	779	55.2	133	0.75	8,8	16,4	15.45	91	
10/9	1655	1343	654	676	50.3	123	0.66	9,0	14,4	15.70	86	

TABLE 3-B

Experimental history. Dog 19–95

EXPERIMENT	DIET	BLOOD REGENERATION		WEIGHT	REMARKS
		Pigment volume	Blood per kilogram		
				kgm.	
Begin 1/16/19	Rice, potato,	1232	100	11.35	Born July 11, 1918
Bled 564 cc.	milk	560	75	11.00	
End 3/7/19		983	90	11.20	Complete regeneration 5 weeks
Begin 3/17/19	Rice, potato,	1092	88	12.80	Table 41
Bled 566 cc.	milk (repeat)	570	75	12.45	
End 4/30/19		1237	98	11.95	Complete regeneration 5 weeks
Begin 8/11/19	Mixed diet	2088	104	13.20	Table 3
Bled 684 cc.		750	74	13.65	
End 10/9/19		1655	86	15.70	Maximum regeneration 7 weeks

TABLE 4

Blood regeneration—mixed diet (following rice, potatoes and milk). Dog 19–96. Bull pup, female, age 6 months

DATE, 1919	PIGMENT VOLUME Hb. PER CENT TIMES BLOOD VOLUME	BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	R. B. C. HEMATOCRIT	Hb.	COLOR INDEX	R. B. C. MILLION	W. B. C.	WEIGHT	BLOOD PER KILOGRAM	REMARKS
		cc.	*cc.*	*cc.*	*per cent*	*per cent*				*kgm.*	*cc.*	
1/16	1200	1025	483	540	52.3	117	0.58	10,1	20,8	10.15	101	
1/16	Diet: Crackermeal and milk											
1/17	Bled 256 cc.											
1/18	Bled 256 cc. No distress											
1/20	514	778	574	196	25.2	66	1.10	3,0	16,0	9.75	80	Normal
1/20	Diet: Boiled rice, 200 grams; potatoes, 200 grams, milk, 500 cc.											
1/27	422	826	604	218	26.4	51	0.48	5,3	12,4	8.60	96	Mild distemper
1/27	Changed to mixed diet*											
2/3	765	915	554	357	39.0	84	0.67	6,3	15,8	10.1	91	Recovered from distemper
2/12	1013	1080	574	500	46.3	94	0.65	7,2	12,0	11.35	95	
2/19	1243	1243	646	584	47.0	100	0.71	7,0	12,8	11.80	105	
2/28	1288	1108	537	554	50.0	116	0.73	8,0	9.2	11.95	93	

* Developed mild case of distemper. Refused to eat rice and potatoes.

TABLE 4-B

Experimental history. Dog 19–96

EXPERIMENT	DIET	BLOOD REGENERATION		WEIGHT	REMARKS
		Pigment volume	Blood per kilogram		
				kgm.	
Begin 1/16/19	Rice, potato,	1200	101	10.15	Born July 14, 1918
Bled 512 cc.	milk for 1	514	80	9.75	
End 2/28/19	week. Mixed diet for 4 weeks	1288	93	11.95	Table 4
Begin 3/17/19	Rice, potato,	1520	92	13.50	Table 44
Bled 620 cc.	milk	527	72	12.40	
End 4/30/19		778	95	9.90	Maximum regeneration weeks
Begin 8/11/19	Mixed diet	1750	98	13.65	Table 5
Bled 668 cc.		650	72	13.35	
End 10/2/19		2012	98	15.45	

Table 5 shows a second experiment on the same dog, 19–96, given in table 4. The first experiment was done at the age of 6 months and this experiment at the age of 13 months. The period of blood regeneration is longer in this experiment as we believe the correct explanation is the low meat content of the mixed diet during this period. This was also

TABLE 5

Blood regeneration—mixed diet. Dog 19–96. Bull pup, female, age 13 months

DATE, 1919	PIGMENT VOLUME = Hb. PER CENT TIMES BLOOD VOLUME	BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	R. B. C. HEMATOCRIT	Hb.	COLOR INDEX	R. B. C. MILLION	W. B. C.	WEIGHT	BLOOD PER KILOGRAM	REMARKS
		cc.	*cc.*	*cc.*	*per cent*	*per cent*				*kgm.*	*cc.*	
8/11	1750	1335	658	670	50.2	131	0.90	7,3	14,6	13.65	98	
8/11	Diet: Bread and milk											
8/12	Bled 334 cc.											
8/13	Bled 334 cc. No distress.											
8/15	650	964	684	275	28.5	67	0.90	3,7	21,6	13.35	72	Normal
8/15	Diet: Mixed diet											
8/21	660	993	686	297	29.9	66	0.80	4,1	12,0	13.9	71	Diet poor in meat
8/28	801	1155	770	368	31.9	69	0.66	5,2	12,4	14.05	82	
9/4	1054	1340	852	474	35.4	79	0.72	5,5	16,2	14.7	91	More meat in diet*
9/11	1255	1340	742	596	43.7	94	0.64	7,3	18,2	14.65	91	
9/18	1348	1225	639	575	46.9	110	0.65	8,5	10,8	14.85	83	
9/25	1511	1280	634	630	49.2	118	0.59	10,0	9,6	14.90	86	
10/2	2012	1517	665	846	55.7	133	0.68	9,8	14,0	15.45	98	

* Poikilocytosis of red cells of moderate degree. Experimental history, table 4–b.

noted in tables 1, 2 and 3, which experiments were all done at the same time. Our experiments are usually carried out in groups of four.

Table 6 illustrates several points. This dog had been under observation for some time under a variety of dietary conditions (see experimental history—table 6-b). A period of sugar feeding gave no blood regeneration (refer to table 16 in the following paper) and 2 weeks of meat feeding had caused a considerable rise in the pigment volume.

The increase in pigment volume brought this figure back to normal in 4 weeks of mixed diet. The blood volume figures are based on determinations made with dry oxalate. This procedure, as has been pointed out, causes a shrinkage of cells and a dilution of the dye which gives blood volume figures abnormally high. Note that the blood volume figures per kilo are 100 to 136 cc. This fact may account for some of the fluctuations in the estimated plasma volume.

TABLE 6

Blood regeneration—mixed diet (following metabolism experiment). Dog 17–28. Bull dog, female, adult

DATE, 1917	PIGMENT VOLUME = Hb. PER CENT TIMES BLOOD VOLUME	BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	R. B. C. HEMATOCRIT	Hb.	COLOR INDEX	R. B. C. MILLION	W. B. C.	WEIGHT	BLOOD PER KILOGRAM	REMARKS
		cc.	*cc.*	*cc.*	*per cent*	*per cent*				*kgm.*	*cc.*	
1/19	1620	1500	600	900	60	108	0.76	7,1	7,4	11.60	129	
	Diet: Sugar diet, January 19 to February 23. Lean meat diet, February 23 to March 10. Mixed diet, March 10 to May 7. (Refer to table 16)											
3/16	1010	1246	735	511	41	81	0.64	6,3	7,2	10.40	119	Good condition
3/23	973	1158	660	498	43	84	0.64	6,5	10,6	9.90	116	
4/13	1910	1645	757	888	54	116	0.71	8,2	22,0	12.30	134	
4/20	1385	1260	630	630	50	110	0.66	8,3	9,8	12.60	100	
5/7	1895	1709	769	940	55	111	0.66	8,4	13,2	12.50	136	Good condition

Blood volume with dry oxalate. Hemoglobin by Sahli tubes.

Table 7 is placed in this paper to give a comparison between the mixed diet experiments and fasting periods. It will be noted that during this fasting period the blood regeneration is very slight. This point will be discussed at length in the next paper.

DISCUSSION

From a review of the tables it is at once obvious that the *pigment volume* and total *red cell volume* after hemorrhage are below the expected values. There are several obscure factors in this reaction which call

TABLE 6-B

Experimental history. Dog 17-28

EXPERIMENT	DIET	BLOOD REGENERATION		WEIGHT	REMARKS
		Pigment volume	Blood per kilogram		
				kgm	
Begin 9/13/16	Bread and milk			8.4	No blood volume data given
Bled 450 cc.				8.4	
End 10/25/16				8.8	Hb. and R.B.C. back to normal
Begin 1/19/17	Sugar and metabolism	1620	129	11.6	Table 16
Bled 750 cc.		549	87	10.4	Lean beef diet 2 weeks followed by mixed diet. Table 6
End 2/23/17		541	117	8.0	
Begin 5/7/17	Sugar + R.B.C., metabolism	1895	136	12.5	Table 76
Bled 854 cc.		594	76	12.0	
End 6/18/17		1085	154	8.3	Put on bread and milk diet
Begin 9/11/17	Sugar 2 weeks	1650	113	14.0	
Bled 794 cc.	Sugar 2 weeks +	751	84	13.5	
End 10/19/17	diamino-acid of gelatin	1040	106	10.0	Beef heart 3 weeks, mixed diet 3 weeks
Begin 6/3/18	Desiccated kidney, bread and milk	1739	73	17.65	
Bled 949 cc.		792	62	16.50	(3 bleedings)
End 6/28/18		1300	74	16.10	
Begin 8/9/18	Sugar and Hb. intraperitoneally	2417	93	17.00	Table 80
Bled 1076 cc.		756	66	15.90	(3 bleedings)
End 11/13/18		2000	106	15.05	
Begin 12/2/18	Lean meat and gelatin	2118	105	15.70	Table 51
Bled 1140 cc.		708	82	15.05	(3 bleedings)
End 1/22/19		1380	99	14.65	
Begin 5/1/19	Fasting	2018	97	15.90	Table 14
Bled 980 cc.		763	71	14.75	(3 bleedings)
End 5/21/19		1007	86	11.50	Found dead. Renal calculi

for discussion. Let us take a type experiment with simple values. A dog with a hemoglobin of 100 per cent, hematocrit of 50 per cent and blood volume of 600 cc. will have a pigment volume of 600. On two successive days the dog is bled one-fourth of the estimated blood volume. Allowing for replacement of plasma which is known to take place, we should expect after these bleedings a hemoglobin of 55 per

TABLE 7

Blood regeneration—fasting. Dog 17–27. White bull mongrel, female, adult

DATE, 1918	PIGMENT VOLUME = Hb. PER CENT TIMES BLOOD VOLUME	BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	R. B. C. HEMATOCRIT	Hb.	COLOR INDEX	R. B. C. MILLION	W. B. C.	WEIGHT	BLOOD PER KILOGRAM	REMARKS
		cc.	*cc.*	*cc.*	*per cent*	*per cent*				*kgm.*	*cc.*	
3/11	2100	1500	735	766	51	140	0.99	7,1	10,4	15.8	95	
3/13	1627	1122	561	561	50	145	1.03	7,0	8,0	15.7	72	*
3/13	Diet: Bread and milk											
3/13	Bled 375 cc. No distress											
3/14	Bled 375 cc. No distress											
3/16	1005	1116	770	346	31	90	1.05	4,3	12,4	15.5	72	*
3/16	Fasting begun											
3/22	1165	1153	726	427	37	101	0.90	5,6	6,8	13.8	76	*
3/29	1086	1075	656	419	39	101	0.67	7,5	6,2	12.3	88	*
4/4	1213	1054	622	433	41	115	0.65	8,8	6,8	11.6	91	*
4/10	1240	1000	620	380	38	124	0.95	6,7	6,2	10.7	93	Excellent condition

* Slight anisocytosis.

Blood volume with dry oxalate. Hemoglobin by Sahli tubes. Experimental history, table 15–b. See below.

cent and total volume of red cells 165 cc. The plasma is constant under such conditions and will be as normal, 300 cc. volume. The blood volume therefore is 465 cc. and pigment volume 256 (465×0.55) which is 43 per cent of the pigment volume at the beginning of the experiment. If there is no reserve red cell influx we should expect a red cell volume of 55 per cent and a pigment volume of 43 per cent normal following two bleedings of one-fourth the total blood volume. The tables

show figures which are consistently below the expected values. This calls for a broad discussion of the various methods of blood volume determination and possible errors inherent in the various methods. We do not wish to review all these points at this time but hope soon to do so in connection with experiments dealing with the actual technique of blood volume determination.

Two possibilities are to be mentioned, however, in this discussion. First the blood volume values may be too high, calculated by the dye method, and the actual bleedings represent more than 50 per cent of the total blood volume. Second, the possibility that the body has the power to modify the ratio of cells to plasma in different parts of the body under normal or abnormal conditions. It is easy to mention these two possibilities but very difficult to adduce experiments which are conclusive. For example in the abnormal condition of shock it seems clear that there is a remarkable disturbance in the ratio of cells to plasma in different parts of the body.

It may not be wise to pursue this question further but best merely to call the reader's attention to the fact that there is a discrepancy between the expected and actual values for red cell volume and pigment volume after unit hemorrhages. This point will be taken up again. The *pigment volume* figures, however, give a clear-cut index of the curve of blood regeneration.

SUMMARY

The term *pigment volume* used in these papers is equivalent to blood volume times per cent hemoglobin. This means the volume of available red cell pigment circulating in the body at the time of blood volume determination.

Given a uniform degree of anemia we may observe the curve of red blood cell regeneration as influenced by a variety of diet factors. Anemia is produced by bleeding the dog one-fourth of the determined blood volume on each of two successive days.

Under these experimental conditions a diet of mixed table scraps will effect complete blood regeneration to normal in a period of 4 to 7 weeks.

Under similar experimental conditions there will be little blood regeneration during a fasting period—mainly a maintenance factor equivalent to the normal daily wastage of red cells.

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BLOOD REGENERATION FOLLOWING SIMPLE ANEMIA

II. Fasting Compared with Sugar Feeding

Analysis of "Sparing Action of Carbohydrates"

G. H. WHIPPLE, C. W. HOOPER AND F. S. ROBSCHEIT

From the George Williams Hooper Foundation for Medical Research, University of California Medical School, San Francisco

Received for publication April 3, 1920

That there is a distinct difference between the blood regeneration during *fasting periods* as compared with *sugar diet periods* comes out from an analysis of the experiments given below (table 25-a). This difference in blood regeneration is not great but is distinctly in favor of the fasting condition,—in other words, *a dog will form more red cells and hemoglobin during a fasting period than during a similar period of sugar feeding.* In neither case is any nitrogenous material taken into the body, so whatever hemoglobin and red cell stroma may be formed must be constructed in the body from body protein or protein split products. The well known "sparing action of carbohydrates" must be considered in the analysis of these experiments given below. It appears that these experiments can be explained most satisfactorily on the basis of a certain protection or sparing of body protein on the part of the sugar, associated with a definite amount of conservation of protein split products.

Having established the curve of blood regeneration which is the result of a mixed diet subsequent to the anemia period, we wish to present experiments to show the type reaction associated with fasting or sugar feeding. In making any analysis of results it is necessary to know how much reserve capacity the normal dog possesses—how much regeneration of red cells or hemoglobin can be effected during periods of fasting or sugar feeding. We must take into consideration too the daily wear and tear of the red cells which is not an accurately established factor. We do not know the life history of the red cell in the normal or anemic dog. We do know the duration of life of the red cell in the normal human being (1), but this life cycle may be different in disease. The

dog's red cells are extremely fragile and this may or may not indicate a shorter life cycle for dog than the established 30-day period for normal human beings. Data on this point are very much to be desired but in their absence we must postulate an unknown factor of red cell or hemoglobin replacement which is present in all our tables.

Any diet, therefore, which is capable of giving a rising curve of blood regeneration following simple anemia is doing two things. The diet is responsible for a replacement of the red cells (3 to 5 per cent per day) which are worn out day by day, as well as the rise in general level of red cell volume above the anemia level. For the sake of analyis we may assume that the replacement value for human beings and dogs is the same—about 3 per cent per day or complete replacement of the total volume of red cells in approximately 30 days. Under most favorable conditions we may see the volume of red cells regenerate from an anemia level of one-third or one-half normal back to 100 per cent within 4 or 5 weeks. To supply a deficit of 50 to 65 per cent of its red cell volume the body requires 30 days or more over and above its maintenance of red cell wastage. This wastage (wear and tear of red cells) in human beings may be 100 per cent in 30 days. This indicates the importance of this replacement factor and further emphasizes the fact that our curves show the reaction of the body *in excess* of this wastage or replacement value.

When we note a falling curve of hemoglobin and red cells after a long diet period we need not hastily postulate hemolysis or blood destruction from some hypothetical toxin. It may be safer to consider the possibility that the body can form no more hemoglobin or red cells to repair the daily wastage. Even the replacement fraction is not being supplied and the curve subsides gradually depending upon the life cycle of the red cells remaining.

EXPERIMENTAL OBSERVATIONS

The experimental procedures have been described in detail in the preceding communication. The majority of these dogs have been under observation in the laboratory since birth and we have studied their reaction to simple anemia following hemorrhage as influenced by a variety of diets. Some of these observations precede and others follow these tabulated fasting or sugar feeding experiments. The experimental histories given with each animal give a review of the many experiments done on the same dog. The value of comparison under these conditions

is greater than obtains in a series of isolated experiments. The dogs were all very fat and well nourished at the beginning of these experiments and were able to tolerate the fasting or sugar periods without disturbance of health or activity.

During the metabolism experiments the dogs were kept in standard metabolism cages constructed with sharp pitch of the cage bottom to insure rapid and complete drainage of urine. The dogs were catheterized every 24 hours and the catheterized specimen, bladder washings

TABLE 8

Blood regeneration—fasting—metabolism—splenectomy. Dog 17–37. White bull mongrel, female, age 10 months

DATE, 1917	PIGMENT VOLUME = Hb. PER CENT TIMES BLOOD VOLUME	BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	R. B. C. HEMATOCRIT	Hb.	COLOR INDEX	R. B. C. MILLION	W. B. C.	WEIGHT	BLOOD PER KILOGRAM
		cc.	*cc.*	*cc.*	*per cent*	*per cent*				*kgm.*	*cc.*
3/26	927	850	459	391	46	109	0.80	6,8	9,4	10.60	80
3/26	Diet: Bread and milk										
3/28	Bled 212 cc.										
3/29	Bled 212 cc.										
3/30	313	626	451	175	28	50	0.68	3,7	15,4	10.40	60
3/30	Fasting begun										
4/2	403	651	475	176	27	62	0.74	4,2	6,4	9.50	70
4/9	492	769	500	269	35	64	0.64	5,0	7,2	8.50	90
4/16	532	729	474	255	35	73	0.66	5,5	5,0	7.70	96
4/23	540	772	502	270	35	70	0.66	5,3	7,8	6.90	112

Blood volume with dry oxalate. Hemoglobin by Sahli tubes.

and cage urine were made up to a uniform volume. A mixed specimen was preserved and duplicate Kjeldahl analyses made. A few cubic centimeters of acetic acid in the cage collection bottle insured an acid reaction in the urine. With care a male or female dog may be catheterized daily without causing any cystitis. After the catheterization the water or sugar solution was given by stomach tube.

Fasting experiments. The two preceding experiments (tables 8 and 9) are to be compared as the experiments were done at the same time.

TABLE 8-A

Total urinary nitrogen—fasting. Dog 17–37

DATE, 1917	TOTAL NITROGEN 24 HOURS URINE	URINE TOTAL 24 HOURS	WEIGHT	REMARKS
	grams	*cc.*	*pounds*	
March 31	2.80	650	21.5	0 feces
April 1	2.30	405	21.1	Trace of feces
2	2.58	433	20.8	0 feces
3	3.19	345	20.6	0 feces
4	2.97	395	20.3	0 feces
5	3.56	418	19.9	Slight diarrhea
6	2.83	460	19.6	0 feces
7	2.88		19.3	0 feces
8	2.49	427	19.0	0 feces
9	2.80	385	18.8	0 feces
10	2.91	473	18.3	0 feces
11	2.69	359	18.3	0 feces
12	2.91	423	17.9	Trace of feces
13	2.60	371	17.6	0 feces
14	2.60	429	17.5	0 feces
15	2.85	411	17.3	0 feces
16	2.86	414	17.1	0 feces
17	2.60	447	16.8	0 feces
18	2.77	458	16.5	0 feces
19	3.19	396	16.3	0 feces
20	3.02	421	16.1	Trace of feces
21	3.42	416	15.8	0 feces
22	3.56	445	15.7	0 feces
23	3.86	471	15.1	0 feces

Dog given 400 cc. of water daily by stomach tube.

TABLE 8-B

Experimenal history—dog 17–37—splenectomy

EXPERIMENT	DIET	BLOOD REGENERATION		WEIGHT	REMARKS
		Pigment volume	Blood per kilogram		
				kgm.	
Begin 12/15/16	Sugar + gelatin	630	66	10.5	
Bled 350 cc.				10.2	
End 1/11/17		.544	84	8.1	Maximum regeneration 2 weeks, then lean meat diet 3 weeks. Mixed diet
Begin 3/26/17	Fastingmetabolism	927	80	10.6	Table 8
Bled 424 cc.		313	60	10.4	Table 73
End 4/23/17		540	112	6.9	Bread, milk, Blaud's pills, 11 weeks. Slight regeneration

Splenectomy 10/23/16.

The dogs are of the same litter but one had been splenectomized. We can make out no difference in the reaction of the splenectomized dog as compared with the control under these experimental conditions. During the fasting period there is a steady rise in hemoglobin, red cell hematocrit, red cell count, total red cell volume and pigment volume. The rise is most pronounced during the first week, as a rule. It is noted that the plasma volume remains constant or decreases slightly. This

TABLE 9

Blood regeneration—fasting—metabolism. Dog 17–38. White bull mongrel, female, age 10 months

DATE, 1917	PIGMENT VOLUME = Hb. PER CENT TIMES BLOOD VOLUME	BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	R. B. C. HEMATOCRIT	Hb.	COLOR INDEX	R. B. C. MILLION	W. B. C.	WEIGHT	BLOOD PER KILOGRAM
		cc.	*cc.*	*cc.*	*per cent*	*per cent*				*kgm.*	*cc.*
3/26	1395	1090	490	600	55	128	0.80	8,0	15,8	9.8	111
3/26	Diet: Bread and milk										
3/28	Bled 272 cc.										
3/29	Bled 272 cc.										
3/30	443	738	531	207	28	60	0.73	4,1	12,2	9.3	79
3/30	Fasting begun										
4/2	532	819	573	246	30	65	0.74	4,4	10,6	8.6	95
4/9	491	723	441	282	39	68	0.65	5,2	9,6	7.5	96
4/16	585	770	439	331	43	76	0.62	6,1	12,0	6.8	113
4/20	585	750	420	330	44	78	0.51	7,6	11,4	6.3	119

Blood volume with dry oxalate. Hemoglobin by Sahli tubes.
Experimental history, see table 20–b.

fact accounts for the increase in blood per kilo, due to loss of body weight.

The nitrogen figures for total urine are of interest. The daily output is fairly constant until we reach the last 4 days of the experiment which show a distinct increase above normal. It is possible that this increase represents the body protein disintegration which may be observed after a long period of fasting, giving rise to the premortal rise in nitrogen. Both these dogs recovered the lost weight promptly when placed upon

a bread and milk diet. There is no evidence for an increase in body protein katabolism to supply the essentials for hemoglobin construction. The same remarks apply to the following experiment (table 10).

The above experiment (table 10) shows a considerable increase in red cells from 5,200,000 to 7,000,000 with an unchanged red cell hematocrit. Poikilocytosis is marked during this period and there is evidence

TABLE 9–A

Total urinary nitrogen—fasting. Dog 17–38

DATE, 1917	TOTAL NITROGEN 24 HOURS URINE	URINE TOTAL 24 HOURS	WEIGHT	REMARKS
	grams	*cc.*	*pounds*	
March 31	3.42	720	19.4	0 feces
April 1	2.16	412	19.1	0 feces
2	2.18	471	18.8	Trace of feces. Vomited
3	2.77	236	18.6	Moderate feces
4	2.74		18.1	0 feces
5	2.94	430	17.9	0 feces
6	2.86	442	17.7	0 feces
7	3.25	428	17.3	Slight diarrhea
8	2.86	422	17.1	0 feces
9	2.66	432	16.7	0 feces
10	2.97	403	16.3	Trace of feces
11	2.46	413	16.2	0 feces
12	2.83	434	15.9	Slight diarrhea
13	2.72	383	15.7	0 feces
14	2.72	443	15.5	0 feces
15	3.05	446	15.1	0 feces
16	3.58	418	14.9	0 feces
17	3.42	408	14.7	0 feces
18	3.25	441	14.4	0 feces
19	4.14	441	14.1	0 feces
20	4.09	726	13.8	0 feces
21	4.17	517	14.3	Slight diarrhea

Dog given 400 cc. water daily by stomach tube.

for a certain amount of fragmentation of the red cells in this and other experiments of similar nature. In some instances there is evidence for a faulty construction of red cells during periods of stress when red cell regeneration is being accomplished with difficulty on a limited diet. It will be possible with the accumulation of much data to ascertain which diets favor stroma construction and which diet factors accelerate hemoglobin construction. It is evident at present that these two fac-

tors do not always run parallel under experimental conditions, as well as in disease.

TABLE 10

Blood regeneration—fasting—metabolism. Dog 16-160. Bull mongrel, female, age 2 years

DATE, 1918	PIGMENT VOLUME = Hb. PER CENT TIMES BLOOD VOLUME	BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	R. B. C. HEMATOCRIT	Hb.	COLOR INDEX	R. B. C. MILLION	W. B. C.	WEIGHT	BLOOD PER KILOGRAM	REMARKS
		cc.	*cc.*	*cc.*	*per cen*	*per cent*				*kgm.*	*cc.*	
5/3		1241	633	590	47.6					10.45	119	
5/3	Diet: Bread and milk											
5/6					45.2	90				10.00		
5/20	1316	1125	523	591	52.5	117	0.70	8,3	14,2	10.60	106	R.B.C.fragment++
5/21	Bled 281 cc.											
5/22	Bled 281 cc.											
5/24	482	752	492	250	33.2	64	0.55	5,8	14,8	10.20	74	
5/25	Fasting begun. Metabolism											
5/31	585	688	455	226	32.8	85	0.82	5,2	6,0	9.10	76	* Poik.
6/5	591	672	437	225	33.5	88	0.72	6,1	6,8	8.40	80	* Poik.++
6/12	636	707	457	243	34.3	90	0.64	7,0	8,2	7.55	94	* Poik.++
6/12	Diet: 200 grams bread and 300 cc. milk. Metabolism discontinued											
6/18	696	791	510	274	34.6	88	0.88	5,0	5,4	8.15	97	* Poik.++
6/26	451	550	360	184	33.5	82	0.68	6,0	9,0	8.20	67	* Slight poik.
6/26	Diet: Changed to mixed diet											

* Poikilocytosis of red cells.

Experimental history, see table 18–b.

This second group of experiments (tables 11, 12 and 13) presents several factors in common. During the fasting period the blood regeneration was notable in two experiments during the first week following the bleeding. In fact this level was scarcely increased during the sub-

sequent fasting period. This rapid increase during the first week may be explained by some reserve factor which is called in during the emergency period. The following weeks show little increase in pigment volume because the body can only supply the material needed to replace the daily wear and tear on the red cells. The third experiment (table 12) shows a more gradual rise in the pigment volume, hematocrit and hemoglobin during the entire fasting period.

TABLE 10–A

Total urinary nitrogen—fasting. Dog 16-160

DATE, 1918	TOTAL NITROGEN 24 HOURS URINE	URINE TOTAL 24 HOURS	WEIGHT	REMARKS
	grams	*cc.*	*pounds*	
May 26	1.76	270	21.8	Some water vomited
27	1.90	120	21.4	About 50 cc. vomited; 0 feces
28	1.85	145	20.8	No vomiting. 0 feces; dog very active
29	2.07	113	20.6	No vomiting. 0 feces
30	2.02	155	20.3	0 feces
31	2.10	188	20.1	0 feces
June 1	2.02	199	19.8	0 feces
2	1.96	134	19.3	0 feces. 300 cc. water
3	2.44	167	19.1	0 feces. Good condition
4	2.16	206	18.9	0 feces
5	2.41	180	18.5	Solid feces
6	2.13	207	18.3	Little feces
7	2.02	221	17.9	0 feces
8	1.79	191	17.6	0 feces
9	1.90	186	17.4	0 feces
10	2.02	181	17.3	0 feces
11	1.90	178	17.0	0 feces
12	2.30	190	16.6	Feces + in urine. Dog in excellent condition
13	1.73	225	16.4	Soft feces

Dog given 200 cc. water daily by stomach tube.

All three experiments show a rapidly developing "dietary deficiency disease" which develops after a period of bread and milk feeding subsequent to the fasting period. This dietary deficiency condition is characterized by ulcerated mucous membranes and much gastro-intestinal disturbance. We are inclined to the opinion that this condition is analogous to scurvy in human beings. In the near future we hope

to report a series of experiments bearing upon this point, making clear the factors concerned in the development of this abnormal condition as well as its cure and prevention in the dog.

TABLE II

Blood regeneration—fasting. Dog 18-103. Brindle bull, female, age 1 year

DATE, 1918	PIGMENT VOLUME = Hb. PER CENT TIMES BLOOD VOLUME	BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	R. B. C. HEMATOCRIT	Hb.	COLOR INDEX	R. B. C. MILLION	W. B. C.	WEIGHT	BLOOD PER KILOGRAM	REMARKS
		cc.	*cc.*	*cc.*	*per cent*	*per cent*				*kgm.*	*cc.*	
8/28	1540	1115	552	547	49.0	138	0.97	7,1	16,0	15.45	72	
8/28	Diet: Crackermeal and milk											
8/28	Bled 279 cc.											
8/29	Bled 279 cc.											
8/31	810	965	650	311	32.2	85				14.95	65	
8/31	Bled 241 cc.											
9/3	590	955	708	242	25.3	62	0.94	3,3	26,0	14.35	65	* Poik.
9/3	Fasting begun											
9/10	946	1076	692	358	33.3	88	0.72	6,1	13,0	13.15	82	
9/16	852	882	552	321	36.4	97	0.88	5,5	11,4	12.15	73	
9/25	745	774	484	275	35.5	96	0.69	7,0	7,2	10.90	71	
9/30	700	784	485	295	37.7	89	0.57	7,8	13,2	10.30	76	Good condition
9/31	Diet: 250 grams crackermeal, 300 cc. milk											
10/11	699	889	572	311	35.0	79				11.30	79	
10/18	692	822	544	274	33.3	84	0.74	5,7	4,8	10.25	76	* Poik. +
10/18	Diet: Mixed diet											
10/22	Death from dietary deficiency disease											

* Poikilocytosis of red cells.

No previous anemia experiments on this dog.

TABLE 12

Blood regeneration—fasting (followed by rice, bread and milk, then yeast). Dog 18–114. White bull, female, adult

DATE, 1919	PIGMENT VOLUME = Hb. PER CENT TIMES BLOOD VOLUME	BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	R. B. C. HEMATOCRIT	Hb.	COLOR INDEX	R. B. C. MILLION	W. B. C.	WEIGHT	BLOOD PER KILOGRAM	REMARKS
		cc.	*cc.*	*cc.*	*per cent*	*per cent*				*kgm.*	*cc.*	
5/1	2550	1830	698	1115	60.9	139	0.75	9,3	12,6	13.95	131	
5/1	Diet: Bread and milk											
5/2	Bled 458 cc.											
5/3	Bled 458 cc. No distress											
5/5	620	1000	690	301	30.1	62	0.86	3,6	14,0	12.60	79	
5/6	Fasting begun											
5/14	890	1043	619	394	37.8	85	0.76	5,6	8,0	11.30	92	* Poik.
5/21	899	968	598	375	38.7	93	0.86	5,4	6,0	10.45	93	* Poik.
5/28	1103	1077	588	478	44.4	102	0.69	7,4	6,4	9.55	108	
5/28	Diet: 200 grams bread, 300 grams rice, 500 cc. milk											
6/4	1166	1166	654	502	43.1	100	0.62	8,1	6,6	10.45	115	* Poik.++
6/5	Diet: 30 grams compressed yeast, 100 grams bread, 500 cc. milk											
6/11	1070	1084	609	464	42.8	99	0.56	8,9	7,4	10.35	105	* Poik++
6/18	1232	1109	572	526	47.4	111	0.57	9,8	6,0	10.30	107	* Poik.†
6/25	1167	1094	547	537	49.1	107	0.54	10,5	10,4	10.25	107	
7/1	1148	1125	594	516	45.9	102	0.65	7,8	11,6	10.00	112‡	
7/1	Diet: Changed to mixed diet. Extra food. Recovery—4 days											

* Poikilocytosis of red cells.

† June 16: Mucous colitis. No yeast given: milk boiled.
June 17: No mucus; 10 grams yeast given; milk boiled.
June 18: No mucus; 20 grams yeast given: 300 cc. fresh milk.

‡ Beginning ulceration of mucous membrane of mouth. Beginning salivation.

Autopsy (see table 14)

Dog 17-28. White bull, female. See experimental history, table 6-b.

May 22, 1919. Found dead and cold. Blood clotted. Left ventricle slightly hypertrophied. Lungs and pleurae negative. Slight hypostatic congestion in right lower lobe. Spleen soft and flabby; normal size; pale pink-gray and cellular. Peritoneal cavity is normal. No pigmentation and no adhesions. Gastro-intestinal tract not opened, but superficially not abnormal. Liver normal size and color; indefinite hazy patches in which lobules look washed out (0.5 to

TABLE 12-B

Experimental history. Dog 18-114

EXPERIMENT	DIET	BLOOD REGENERATION		WEIGHT	REMARKS
		Pigment volume	Blood per kilogram		
				kgm.	
Begin 4/24/18	Cooked liver, bread, and milk	553	94	7.85	Slight anemia
Bled 279 cc.		435	105	7.65	Table 63
End 5/29/18		791	84	9.60	Maximum regeneration 2 weeks. Mixed diet
Begin 8/14/18	Powdered liver, cracker-meal and milk	1773	106	12.20	
Bled 816 cc.		770	95	11.75	(3 bleedings)
End 9/27/18		2190	112	12.90	
Begin 11/14/18	Cooked liver only	1640	105	13.50	Table 61
Bled 976 cc.		592	81	12.50	(3 bleedings)
End 12/11/18		1902	111	13.65	Complete regeneration 3 weeks
Begin 5/1/19	Fasting	2550	131	13.95	Table 12
Bled 916 cc.		620	79	12.60	
End 5/28/19		1103	108	9.55	

2 cm. in diameter). Bladder contains a little syrupy pus-like urine; slight cystitis. Left kidney large and hard; few cysts on surface; large stone fills pelvis (2 x 10 cm. ±). Right kidney shrunken to small size (2 x 3 x 5 cm.); stone in pelvis. Marrow of femur almost all fat. Marrow of rib is normal. Pancreas is small and warty looking—chronic change involves all of lower arm and head, all but distal fourth of upper arm. No evidence of acute process. Ovaries negative. Uterus shows sites of placental attachment. Urine: obtained from bladder at post-mortem. Small amount, mostly pus. No sugar. Few hyaline casts. Many pus cells and small round epithelial cells, few partially destroyed red cells. Bacteria abundant.

The experiment given in table 14 is complicated by nephritis, pyelitis and bilateral renal calculi. The blood regeneration during the two weeks preceding death was normal in every way. If anything, the

TABLE 13

Blood regeneration—fasting (followed by rice and bread and yeast vitamine). Dog 18–116. White bull, female, adult

DATE, 1919	PIGMENT VOLUME = Hb. PER CENT TIMES BLOOD VOLUME	BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	R. B. C. HEMATOCRIT	Hb.	COLOR INDEX	R. B. C. MILLION	W. B. C.	WEIGHT	BLOOD PER KILOGRAM	REMARKS
		cc.	*cc.*	*cc.*	*per cent*	*per cent*				*kgm.*	*cc.*	
5/1	2620	1930	852	1050	54.4	133	0.85	7,8	8,0	18.75	103	
5/1	Diet: Bread and milk											
5/2	Bled 483 cc.											
5/3	Bled 483 cc. No distress											
5/5	778	1245	884	355	28.5	62	0.79	3,9	12,8	17.45	71	
5/6	Fasting begun											
5/14	1178	1357	818	525	38.7	87	0.84	5,2	14,0	15.95	85	* Poik.
5/21	1025	1205	742	434	36.0	85	0.75	5,7	9,4	14.85	81	* Poik.++
5/28	1176	1233	720	519	40.7	95	0.73	6,5	7,6	13.85	89	* Poik.++
5/28	Diet: 300 grams rice, 200 grams bread											
6/4	1109	1265	798	462	36.5	88	0.62	7,1	9,8	14.75	86	* Poik.++
6/5	Diet: 1 gram yeast vitamine,† 100 grams bread, 500 cc. milk											
6/11	1065	1232	761	458	37.2	86	0.70	6,1	8,2	14.65	84	* Poik.+
6/18	1368	1323	730	580	43.8	103	0.54	9,6	10,2	14.60	91	* Poik.+
6/25	1280	1380	780	594	43.0	93	0.51	9,2	5,6	14.05	98	* Poik.++
6/25	Diet: Changed to mixed diet. Developed dietary deficiency disease											
6/30	Death											

* Poikilocytosis of red cells.

† Vitamine prepared according to Seidell's method (2).

blood pigment production was above the average. This is of some interest because of the frequent occurrence of anemia in human beings associated with advanced chronic nephritis. This dog's death was

certainly due to renal injury and insufficiency, but the picture was not that of a primary essential chronic nephritis. In this single instance the development of a subacute renal disease did not impair the function of the organs of the body which are responsible for blood and hemoglobin regeneration.

Sugar feeding experiments. The first three experiments are similar and give strong evidence to show that there is very little regeneration of red cells and hemoglobin during a sugar feeding period. The reac-

TABLE 13-B

Expermiental history. Dog 18-116

EXPERIMENT	DIET	BLOOD REGENERATION		WEIGHT	REMARKS
		Pigment volume	Blood per kilogram		
				kgm.	
Begin 4/24/18	Meat extract,	903	99	9.75	Table 65
Bled 540 cc.	bread and	422	89	9.65	(3 bleedings)
End 5/29/18	milk	865	89	10.85	Mixed diet 2 weeks. Complete regeneration
Begin 8/14/18	Thyroid,	1842	95	14.20	
Bled 944 cc.	crackermeal	1055	98	14.25	(3 bleedings)
End 10/24/18	and milk	1640	114	11.85	Complete regeneration 7 weeks
Begin 12/2/18	Beef heart	2120	115	14.90	Table 52
Bled 1160 cc.	and liver	776	90	13.95	(3 bleedings)
End 12/30/18		2270	106	15.50	
Begin 5/1/19	Fasting	2620	103	18.75	Table 13
Bled 966 cc.		778	71	17.45	
End 5-28-19		1176	89	13.85	

tion is constantly in favor of the fasting period during which time the dog can make a definite gain in red cells and hemoglobin, in excess of the maintenance requirements. In the first two experiments (tables 15 and 16) we have control observations in the same dogs during fasting periods (tables 7 and 14). The regeneration is distinctly more during fasting. In all three experiments the gain in pigment volume is present in the first week and we may wish to assume some emergency reserve to account for this reaction. The following weeks show little

or no subsequent gain and may even show a falling off which indicates that the body cannot fabricate sufficient hemoglobin and red cells for its daily needs.

Splenectomy (table 17) done some time before this experiment does not appear to modify the reaction of the red cells and hemoglobin under the conditions of these experiments.

TABLE 14

Blood regeneration—fasting—nephritis and renal calculi. Dog 17-28. White bull, female, adult

DATE 1919	PIGMENT VOLUME = Hb. PER CENT TIMES BLOOD VOLUME	BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	R. B. C. HEMATOCRIT	Hb.	COLOR INDEX	R. B. C. MILLION	W. B. C.	WEIGHT	BLOOD PER KILOGRAM	REMARKS
		cc.	*cc.*	*cc.*	*per cent*	*per cent*				*kgm.*	*cc.*	
5/1	2018	1550	706	829	53.5	130	0.76	8,6	7,0	15.9	97	
5/2	Diet: Bread and milk											
5/2	Bled 390 cc.											
5/3	Bled 390 cc. No distress											
5/5	763	1040	686	349	33.5	73	1.14	3,2	9,4	14.75	71	
5/6	Bled 200 cc. No distress											
5/8	631	956	648	295	30.8	66	0.94	3,5	8,2	14.25	67	
5/8	Fasting begun											
5/14	903	1041	643	415	39.9	87	0.79	5,5	7,8	13.0	80	
5/21	1007	988	535	432	43.8	102	0.74	6,9	8,2	11.5	86	*
5/22	Found dead. Autopsy given below											

* Dog is sick, weak and thirsty; distended abdomen.

Experimental history, see table 6-b.

The three metabolism tables (tables 15-a, 16-a and 17-a) in general show the characteristic reaction in urinary nitrogen. In every instance at the beginning when the anemia reaction is most intense we note the expected drop in urinary nitrogen when sugar is administered. The "sparing action of carbohydrate" is not disturbed by the presence of this degree of secondary anemia. The first and second experiments

show a distinct rise in urinary nitrogen associated with the bleeding periods. These dogs showed considerable distress during the second bleeding period and it is highly probable that more than one-fourth of the total blood volume was removed on these two days (note the high figure, 130 cc. per kgm., for blood volume as determined by the dry oxalate method). We are inclined to attribute this rise in urinary nitrogen to the shock of the bleeding—to the tissue injury produced by

TABLE 15

Blood regeneration—sugar feeding—metabolism. Dog 17-27. White bull mongrel, female, adult

DATE 1917	PIGMENT VOLUME = Hb. PER CENT TIMES BLOOD VOLUME	BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	R. B. C. HEMATOCRIT	Hb.	COLOR INDEX	R. B. C. MILLION	W. B. C.	WEIGHT	BLOOD PER KILOGRAM	REMARKS
		cc.	*cc.*	*cc.*	*per cent*	*per cent*				*kgm.*	*cc.*	
1/19	1630	1507	603	904	60.0	108	0.71	7,6	9,0	11.60	130	Fasting
1/20	Bled 375 cc.											
1/22	Bled 375 cc.											
1/24	598	950	627	323	34.0	63	0.73	4,3	8,2	10.50	91	
1/24	Diet: 50 grams cane sugar, 25 grams glucose											
2/2	806	1203	698	506	42.0	67	0.56	6,0	6,0	9.6	125	* Anis. ++
2/9	763	1090	643	447	41.0	70	0.57	6,1	8,8	9.0	121	* Anis. ++
2/14	750	1028	596	432	42.0	73	0.70	5,2	7,6	8.2	125	Dog refuses sugar

* Anisocytosis of red cells

Blood volume with dry oxalate. Hemoglobin by Sahli tubes.

Compare fasting period, table 7.

the hemorrhage, and this observation is in harmony with those of Buell (3) working with pigs. Our third experiment (table 17-a) shows little or no increase in urinary nitrogen, no clinical reaction and a low estimated blood volume (80 cc. per kgm.).

After the first exhibition of sugar we note a uniform low level of urinary nitrogen excretion. This low level is constant until the end of the experiments except in the first (table 15-a). Here we note a rise in urinary nitrogen during the last five days. It is fair to say that this

TABLE 15-A

Total urinary nitrogen—sugar. Dog 17-27

DATE, 1917	TOTAL NITROGEN 24 HOURS URINE	URINE TOTAL 24 HOURS	WEIGHT	REMARKS
	grams	*cc.*	*pounds*	
January 17	3.25	467	25.9	Fasting
18	2.58	411	26.0	
19	2.58	461	25.6	
20	2.86	422	25.1	Bled 375 cc.
21	3.75	381	24.1	0 feces
22	4.09	330	24.0	Bled 375 cc. Very weak
January 23	Anemia period begun—Diet: 50 grams sugar, 25 grams glucose			
23	3.30	537	23.8	0 feces
24	3.39	511	23.1	Diarrhea
25	1.99	427	22.8	0 feces
26	1.74	367	22.6	0 feces
27	2.02	390	22.3	Slight diarrhea
28	2.69	430	22.2	0 feces
29	2.38	435	21.9	0 feces
30	2.63	404	21.9	0 feces
31	1.74	401	21.7	0 feces
February 1	1.62	417	21.6	0 feces
2	1.46	401	21.2	0 feces
3	1.62	459	20.9	0 feces
4	1.96	375	0.8	Feces+
5	1.76	377	20.6	0 feces
6	2.07	400	20.5	0 feces
7	lost		20.4	0 feces
8	1.60	520	20.2	0 feces
9	1.90	475	19.8	Diarrhea
10	2.35	482	19.3	Diarrhea
11	3.86	720	18.4	0 feces. Vomitus +. Dog is sick
12	4.76	568	17.7	Vomitus +. Refuses sugar solution
13	4.76	361	18.1	0 feces
14	3.67	541	18.0	0 feces
15	4.20	505	17.8	Diarrhea +. Milk diet

Dog given 400 cc. water daily by stomach tube.

dog was sick, vomited the sugar solution and had diarrhea. This period is properly considered as an interval of intoxication in which little sugar was retained and perhaps this amount favored the intestinal irritation which was conspicuous.

TABLE 15-B

Experimental history. Dog 17-27

EXPERIMENT	DIET	BLOOD REGENERATION		WEIGHT	REMARKS
		Pigment volume	Blood per kilogram		
				kgm.	
Begin 9/13/16	Lean meat			8.3	
Bled 450 cc.				8.9	(3 bleedings)
End 10/24/16				11.4	Complete regeneration of Hb. and R.B.C.
Begin 1/19/17	Sugar, metabolism	1628	130	11.6	Table 15
Bled 350 cc.		598	91	10.5	
End 2/16/17		646	119	8.6	Bread and milk 5 months. Slight regeneration
Begin 10/17/17	Sugar, metabolism	2150	123	13.4	
Bled 826 cc.	Sugar, gelatin, metabolism	718	84	13.2	
End 11/24/17		915	108	10.1	Sugar, gelatin, crackermeal, lard and butter, 3 weeks. Dietary deficiency disease. Recovery 2 weeks
Begin 3/13/18	Fasting	1626	72	15.7	Table 7
Bled 750 cc.		1004	72	15.5	
End 4/10/18		1240	93	10.7	
Begin 6/3/18	Cooked thymus, bread and milk	1939	87	16.35	
Bled 1022 cc.		723	70	15.75	(3 bleedings)
End 6/28/18		1245	78	15.70	
Begin 8/9/18	Hemoglobin intravenously. Sugar	1914	90	16.15	Table 79
Bled 988 cc.		817	67	15.45	(3 bleedings)
End 8/30/18		1155	80	13.75	Killed September 3

Tables 18 and 18-b deserve special mention as they show the results of several experiments done under controlled conditions on the same dog. The experimental history refers to table 10, which shows the blood regeneration on this dog during a fasting period. There is a dis-

tinct increase in pigment volume, hematocrit and hemoglobin during the fasting period. We see in table 18 the same dog under identical conditions on a sugar diet. During the same interval we note practically the same value for pigment volume, hematocrit and hemoglobin at the beginning and end of the sugar feeding. There is a trifling rise during the first week but this is subsequently lost with return to the initial level. One of the tables to follow (table 21) shows the same dog on a sugar and

TABLE 16

Blood regeneration—sugar feeding—metabolism. Dog 17–28. White bull, female, adult

DATE, 1917	PIGMENT VOLUME = Hb. PER CENT TIMES BLOOD VOLUME	BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	R. B. C. HEMATOCRIT	Hb.	COLOR INDEX	R. B. C. MILLIONS	W. B. C.	WEIGHT	BLOOD PER KILOGRAM	REMARKS
		cc.	*cc.*	*cc.*	*per cent*	*per cent*				*kgm.*	*cc.*	
1/19	1620	1500	600	900	60.0	108	0.76	7,1	7,4	11.60	129	Fasting
1/20	Bled 375 cc.											
1/22	Bled 270 cc.											
1/23	Bled 105 cc.											
1/24	588	900	603	306	34.0	61	0.80	3,8	7,8	10.40	87	
1/24	Diet: 50 grams cane sugar, 25 grams glucose, 400 cc. water											
2/2	717	1121	684	437	39.0	64	0.62	5,2	7,2	9.40	120	* Anis.
2/9	636	1027	637	390	38.0	62	0.65	4,8	6,2	8.90	115	
2/16	634	961	586	375	39.0	66	0.59	5,6	10,0	8.50	113	Diarrhea +
2/23	541	933	562	373	40.0	58	0.56	5,2	9,0	8.00	117	

* Anisocytosis of red cells.

Blood volume with dry oxalate. Hemoglobin by Sahli tubes.

Experimental history, see table 6-b.

gliadin diet which holds the pigment volume curve about on a level—no gain nor loss.

Table 19 is unsatisfactory from the standpoint of the blood volume and pigment volume figures but in view of the other experiments we venture to include it because this dog illustrates a reaction which is not uncommon in dogs bled for the first time. In dogs used for the first time in anemia experiments we often note a remarkable regeneration of

TABLE 16-A

Total urinary nitrogen—sugar. Dog 17–28

DATE. 1917	TOTAL NITROGEN 24 HOURS URINE	URINE TOTAL 24 HOURS	WEIGHT	REMARKS
	grams	*cc.*	*pounds*	
January 17	2.46	482	25.9	Fasting
18	2.10	340	25.8	
19	2.46	425	25.6	Diarrhea
20	2.21	519	24.8	Bled 373 cc.
21	2.80	375	23.6	
22	3.08	320	23.6	Bled 270 cc. Dyspnoea
23	2.49	493	23.6	Bled 105 cc.
January 24	Anemia period begun. Diet: 50 grams sugar, 25 grams glucose			
24	2.46	495	22.8	
25	1.96	470	22.4	0 feces
26	1.99	432	22.3	0 feces
27	2.86	427	22.1	0 feces
28	2.69	440	21.8	0 feces
29	2.07	446	21.5	Diarrhea +
30	2.04	406	21.3	0 feces
31	1.71	398	21.2	0 feces
February 1	1.62	391	20.9	
2	1.79	396	20.6	Trace feces
3	1.60	452	20.4	0 feces
4	1.74	407	20.3	0 feces
5	1.68	395	20.1	0 feces
6	1.23	383	20.0	0 feces
7			19.9	0 feces
8	1.74	425	19.7	Feces +
9	1.48	398	19.6	
10	1.51	415	19.4	0 feces
11	1.43	356	19.3	0 feces
12	1.40	387	19.2	0 feces
13	1.48	394	19.1	0 feces
14	1.51	388	18.9	0 feces
15	1.40	391	18.8	0 feces
16	1.26	390	18.6	Diarrhea +
17	1.51	378	18.4	0 feces
18	1.15	390	18.3	0 feces
19	1.23	399	18.1	0 feces
20	1.40	385	18.1	0 feces
21	1.43	409	17.9	0 feces
22	1.20	384	17.8	0 feces
23	1.12	346	17.6	0 feces
24	1.32	374	17.5	

Dog given 400 cc. water daily by stomach tube.

red cells and hemoglobin on unfavorable diets. This was not understood at first but it may be stated as a fact to be explained or not. It is possible that this type of dog has a greater reserve stored in its body from which it can construct red cells and hemoglobin on demand but a dog which has been bled at various times has not this large reserve. At any rate, this dog was able to give a remarkable exhibition of regeneration of red cells and hemoglobin during a short period of sugar feeding. The red cell hematocrit rose from 26 per cent to 47 per cent and

TABLE 17

Blood regeneration—sugar feeding—metabolism—splenectomy. Dog 17–34. White bull mongrel, female, adult

DATE, 1917	PIGMENT VOLUME = Hb PERCENT TIMES BLOOD VOLUME	BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	R. B. C. HEMATOCRIT	Hb.	COLOR INDEX	R. B. C. MILLION	W. B. C.	WEIGHT	BLOOD PER KILOGRAM	REMARKS
		cc.	*cc.*	*cc.*	*per cent*	*per cent*				*kgm.*	*cc.*	
1/19	886	883	459	424	48.0	103	0.69	7,3	14,8	10.9	81	Fasting
1/20	Bled 221 cc.											
1/22	Bled 221 cc.											
1/24	414	780	546	234	30.0	53	0.80	3,3	11,4	10.1	77	
1/24	Diet: 50 grams cane sugar, 25 grams glucose, 400 cc. water											
2/2	528	979	676	303	31.0	54	0.60	4,5	6,4	9.3	105	
2/9	536	893	607	286	32.0	60	0.62	4,8	6,2	8.8	101	* Anis.
2/16	497	888	604	284	32.0	56	0.57	4,9	7,8	8.3	107	
2/23	556	868	567	304	35.0	64	0.60	5,3	6,4	7.8	113	

* Anisocytosis of red cells.

Blood volume with dry oxalate. Hemoglobin by Sahli tubes.

hemoglobin from 62 to 121 per cent. This regeneration was almost completed during 4 weeks on a diet which is least favorable to blood regeneration.

Tables 20, 20-a and 20-b present data on sugar feeding which support the other experiments given above. This dog was observed during a fasting period (table 9) as well as during this sugar period and subsequently during other diet periods of hemoglobin regeneration. This experiment shows only a 2-week period of pure sugar feeding during

TABLE 17-A

Total urinary nitrogen—sugar. Dog 17-34

DATE, 1917	TOTAL NITROGEN 24 HOURS URINE	URINE TOTAL 24 HOURS	WEIGHT	REMARKS
	grams	*cc.*	*pounds*	
January 17	3.42	303	25.0	Fasting
18	3.30	424	24.4	Diarrhea +
19	2.63	406	24.0	
20	2.52	412	23.6	0 feces. Bled 221 cc.
21	2.58	326	22.9	
22	2.86	316	22.8	Bled 221 cc.
January 23	Anemia period begun. Diet: 50 grams sugar, 25 grams glucose			
23	2.66	526	22.8	Trace soft feces
24	2.35	436	22.2	
25	1.99	371	22.0	Trace feces
26			21.8	0 feces
27	2.46	351	21.6	Slight diarrhea
28	2.69	431	21.3	0 feces
29	2.60	371	21.2	0 feces
30	2.77	397	21.0	Trace of feces
31	1.68	382	20.8	0 feces
February 1	1.54	396	20.6	0 feces
2	1.46	396	20.4	
3	1.74	381	20.2	0 feces
4	1.63	426	20.0	0 feces
5	1.54	341	20.4	0 feces
6	1.51	406	19.8	0 feces
7			19.6	Diarrhea +
8	1.51	420	19.6	0 feces
9	1.48	406	19.3	
10	1.34	386	19.0	0 feces
11	1.51	380	18.8	0 feces
12	1.34	361	18.9	0 feces
13	1.60	412	18.7	0 feces
14	1.40	401	18.4	Trace of feces
15	1.40	381	18.4	0 feces
16	1.40	386	18.3	0 feces
17	1.37	396	18.0	Trace of feces
18	1.46	376	17.8	Trace of feces
19	1.46	432	17.7	0 feces
20	1.48	367	17.7	Trace of feces
21	1.34	411	17.6	0 feces
22	1.48	391	17.4	0 feces
23	1.62	431	17.1	0 feces
24	1.74	384	17.13	Diarrhea. Boiled milk diet

Dog given 400 cc. water daily by stomach tube.

TABLE 17-B

Experimental history. Dog 17–34. Splenectomy

EXPERIMENT NUMBER	DIET	BLOOD REGENERATION		WEIGHT	REMARKS
		Pigment volume	Blood per kilogram		
				kgm.	
Begin 12/15/16	Meat and			10.9	No blood volume
Bled 390 cc.	bread only				data
End 1/11/17		972	85	11.3	Complete regenera-
				12.5	tion of Hb. and R. B. C.
Begin 1/20/17	Sugar.	910	81	10.9	Table 17
Bled 442 cc.	Metabolism	413	77	10.1	
End 2/23/17		556	113	7.8	Bread and milk diet 5 months
Begin 6/3/18	Powdered	1331	63	16.6	
Bled 807 cc.	liver, bread	664	62	15.7	(3 bleedings)
End 6/28/18	and milk	960	70	15.3	

Splenectomy 10/3/16.

TABLE 18

Blood regeneration—sugar feeding. Dog 16–160. Bull mongrel, female, age 2 years +

DATE, 1918	PIGMENT VOLUME = Hb. PER CENT TIMES BLOOD VOLUME	BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	R. B. C. HEMATOCRIT	Hb.	COLOR INDEX	R. B. C. MILLION	W. B. C.	WEIGHT	BLOOD PER KILOGRAM	REMARKS
		cc.	*cc.*	*cc.*	*per cent*	*per cent*				*kgm.*	*cc.*	
8/28	1835	1103	456	654	58.2	166	1.1	7,6	9,6	10.15	109	* Slight poik.
8/28	Diet: Crackermeal and milk											
8/28	Bled 276 cc.											
8/29	Bled 276 cc.											
8/31	648	771	524	243	31.5	84				9.55	81	
9/1	Bled 193 cc.											
9/3	448	696	513	177	25.5	64	0.8	4,0	15,4	9.5	73	* Poik.+
9/3	Diet: 75 grams sugar, 25 grams dextrose by stomach tube											
9/10	561	792	577	211	26.7	71	0.96	3,7	9,4	8.65	92	* Poik.++
9/16	488	703	500	192	27.3	69	0.78	4,4	7,8	8.0	88	* Poik.++
9/25	429	664	474	182	27.4	64	0.61	5,2	9,6	7.35	90	* Poik.++

* Poikilocytosis of red cells.

For continuation of experiment see table 80.

TABLE 18-B

Experimental history. Dog 16–160

EXPERIMENT NUMBER	DIET	BLOOD REGENERATION		WEIGHT	REMARKS
		Pigment volume	Blood per kilogram		
				kgm.	
Begin 9/26/16	Mixed			5.70	No blood volume data
Bled 440 cc.				6.10	(4 bleedings)
End 11/29/16				7.30	Complete regeneration of Hb. and R. B. C.
Begin 2/12/17	Bread, milk and Blaud's pills	938	127	7.70	Table 68
Bled 488 cc.		423	80	7.40	
End 7/16/17		456	97	5.90	Maximum regeneration 17 weeks
Begin 10/17/17	Sugar and gliadin	1278	122	8.90	Metabolism
Bled 542 cc.		599	95	8.90	Table 21
End 12/3/17		755	106	6.30	Crackermeal, gelatin, lard, butter, 2 weeks. Mixed diet 5 weeks. Complete regeneration
Begin 5/20/18	Fasting	1316	106	10.60	Metabolism
Bled 562 cc.		482	74	10.20	Table 10
End 6/12/18		636	94	7.55	Bread and milk 3 weeks
Begin 8/28/19	Sugar 3 weeks.	1835	109	10.15	Table 18
Bled 745 cc.	Sugar and Hb.	448	73	9.50	(3 bleedings)
End 10/16/19	intravenously 1 week	586	95	7.80	Dried yeast and crackermeal, 2 weeks. Slight regeneration. Table 80
Begin 2/20/19	Sugar and carrot	1375	99	11.30	Table 22
Bled 781 cc.	juice	548	81	10.10	(3 bleedings)
End 3/18/19	Dried yeast, bread and milk Beef liver, bread and milk	474	86	8.75	
Begin 8/8/19	Beet tops, bread	1220	97	10.80	
Bled 526 cc.	and milk	600	66	13.15	
End 12/5/19	Spinach, bread and milk Compressed yeast, spinach, bread and milk	799	95	8.60	

which time is recorded a moderate increase in red cells and pigment volume. This increase is somewhat above the average reaction under similar conditions and if sugar feeding had been continued for 2 more weeks a drop in hemoglobin, pigment volume and red cell hematocrit was to be expected.

Histidine (1.5 gram per day) added to this diet actually prevented the expected fall and is responsible for a *definite gain* in the first week of

TABLE 19

Blood regeneration—sugar feeding. Dog 19–28. Fox terrier, female, adult

DATE, 1918	PIGMENT VOLUME = Hb. PER CENT TIMES BLOOD VOLUME	BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	R. B. C. HEMATOCRIT	Hb.	COLOR INDEX	R. B. C. MILLION	W. B. C.	WEIGHT	BLOOD PER KILOGRAM	REMARKS
		cc	*cc.*	*cc.*	*per cent*	*per cent*				*kgm.*	*cc.*	
9/18	1193	790	341	441	55.8	151	0.94	8,0	8,4	8.35	94	
9/18	Diet: Crackermeal and milk											
9/20	Bled 198 cc.											
9/21	Bled 178 cc. Dyspnoea											
9/23	316	509	370	137	26.9	62	0.80	3.9	22,6	7.85	65	
9/23	Diet: 50 grams sugar daily with 150 cc. water											
10/2	637	637	349	284	44.5	100	0.81	6,2	8,6	6.90	92	
10/11					47.2	107				6.10		
10/18	562	464	242	219	47.3	121	0.89	6,8	7,8	5.75	81	*

* Hemolysis in tubes containing blood and dye. Reading of color unsatisfactory.

No previous anemia experiments on this dog.

histidine feeding. In view of the constancy of the sugar feeding reaction in the third week of blood regeneration we attach considerable importance to this reaction. We feel that histidine may be in part concerned in the complicated endogenous reaction which is responsible for the final elaboration of the complex protein hemoglobin.

The last 2 weeks of histidine feeding show merely a level curve of hemoglobin and pigment volume indicating that the maintenance factor alone is being supplied. Under sugar feeding alone a slowly fall-

ing curve would be expected, so that the reaction as a whole is strongly in favor of the histidine feeding as being a contributory factor in the hemoglobin regeneration under these experimental conditions. The small amount of histidine given is significant when we consider the percentage content of histidine in casein (2.5 per cent). It will be noted in a subsequent paper that casein is not an efficient food for hemoglobin regeneration.

TABLE 20

Blood regeneration—sugar and histidine—metabolism. Dog 17–38. White bull mongrel, female, young adult

DATE, 1917	PIGMENT VOLUME = Hb PER CENT TIMES BLOOD VOLUME	BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	R. B. C. HEMATOCRIT	Hb.	COLOR INDEX	R. B. C. MILLION	W. B. C.	WEIGHT	BLOOD PER KILOGRAM
		cc.	*cc.*	*cc.*	*per cent*	*per cent*				*kgm.*	*cc.*
9/11	1342	1231	505	726	59.0	109	0.59	9,3	14,0	11.60	106
9/11	Diet: Bread and milk										
9/12	Bled 308 cc.										
9/13	Bled 308 cc.										
9/15	468	884	619	265	30.0	53	0.68	3,9	12,8	11.30	78
9/15	Diet: 50 grams cane sugar, 25 grams dextrose, 300 cc. water										
9/21	572	805	550	290	36.0	71	0.71	5,0	11,8	10.30	78
9/28	574	755	468	287	38.0	76	0.58	6,5	6,6	9.60	79
10/1	Diet: 50 grams cane sugar, 25 grams dextrose, 1½ gram histidine, 300 cc. water										
10/5	668	795	468	326	41.0	84	0.68	6,2	8,4	8.90	76
10/12	735	826	471	355	43.0	89	0.58	7,7	7,2	8.00	103
10/19	684	786	456	330	42.0	87	0.60	7,3	9,2	7.30	107

Blood volume with dry oxalate. Hemoglobin by Sahli tubes.

The total nitrogen elimination is uniform and sustained at the expected low level except during the last week of the experiment when a decided increase is noted. This type of intoxication is not infrequent after long sugar diet periods and we believe is of gastro-intestinal origin. It is a fact that the hemoglobin and pigment volume show no increase during this period of increased protein katabolism but rather

TABLE 20-A

Total urinary nitrogen—sugar and histidine. Dog 17–38

DATE, 1917	TOTAL NITROGEN 24 HOURS URINE	URINE TOTAL 24 HOURS	WEIGHT	REMARKS
September 15	Diet: 50 grams cane sugar, 25 grams dextrose, 300 cc. water			
	grams	*cc.*	*pounds*	
16	2.52	415	23.9	Diarrhea. Vomited 55 cc.
17	1.93	370	23.7	Diarrhea. Vomited
18	1.46	401	23.4	Soft feces. Vomited 100 cc.
19	1.40	315	23.2	Vomited 50 cc.
20	1.34	240	23.1	0 feces
21	1.65	290	22.5	Diarrhea
22	1.23	285	22.5	0 feces
23	1.34	346	22.1	Slight diarrhea
24	1.34	247	21.8	0 feces
25	1.28	271	21.8	Slight diarrhea. Vomited
26	1.40	275	21.4	0 feces. Vomited 45 cc.
27	1.29	225	21.4	Soft feces. Vomited 25 cc.
28	1.20	245	21.2	Slight diarrhea
29	1.29	336	20.8	Soft feces
30	1.29	251	20.7	0 feces
October 1	Diet: 50 grams cane sugar, 25 grams dextrose, 1.5 gram histidine. 300 cc. water			
October 1	1.15	317	20.5	Feces +
2	1.23	290	20.5	0 feces
3	1.57	303	20.2	Slight diarrhea
4	1.34	264	19.9	0 feces. Vomited
5	1.65		19.6	
6	1.48	152	19.5	0 feces. Vomited 55 cc.
7	1.62	620	18.6	0 feces. Vomited
8	1.85	416	18.3	0 feces. Vomited
9	1.68	111	18.6	
10	2.63	401	18.1	0 feces
11	2.35	261	18.3	Vomited
12	3.25	453	17.6	0 feces. Vomited
13	3.08	341	17.6	0 feces
14	3.14	300	17.4	0 feces
15	2.86	415	17.1	0 feces
16	2.88	266	16.9	0 feces
17	2.58	329	16.8	0 feces
18	2.24	308	16.3	Moderate diarrhea
19	1.68	281	16.1	Diarrhea. Fair condition

TABLE 20-B

Experimental history. Dog 17–38

EXPERIMENT NUMBER	DIET	BLOOD REGENERATION		WEIGHT	REMARKS
		Pigment volume	Blood per kilogram		
				kgm.	
Begin 12/18/16	Sugar and gelatin			8.60	No blood volume data
Bled 600 cc.				8.20	(4 bleedings)
End 1/11/17		532	93	7.00	Slight regeneration of Hb. and R. B. C.
Begin 3/26/17	Fasting, metabolism	1395	111	9.80	Table 9
Bled 544 cc.		443	79	9.30	
End 4/20/17		585	119	6.30	Bread and milk 3 months
Begin 9/11/17	Sugar	1341	106	11.60	Table 20
Bled 616 cc.	Sugar and histidine	468	78	11.30	
End 10/19/17	Metabolism	684	107	7.30	Beef heart followed by mixed diet
Begin 6/3/18	Gelatin, bread and milk	1621	86	12.15	
Bled 753 cc.		723	76	11.25	(3 bleedings)
End 6/28/18		1071	85	10.70	Mixed diet
Begin 8/8/18	Cooked brain, crackermeal and milk	2060	109	11.65	
Bled 825 cc.		526	73	11.20	(3 bleedings) Pregnant
End 9/27/18		1100	84	11.95	Maximum regeneration 3 weeks
Begin 2/6/19	Bread (343 grams) milk (200 cc.)	1873	117	12.15	Table 26
Bled 710 cc.		520	77	11.10	
End 3/19/19		1092	95	11.35	Maximum regeneration 3 weeks
Begin 3/31/19	Bread (100 grams) milk (500 cc.)	1275	96	12.00	Table 26
Bled 580 cc.		499	78	11.40	
End 4/9/19		691	91	10.00	Maximum regeneration 4 weeks

a slight loss in red cell hematocrit and pigment volume. This will be shown to be true in other *abnormal conditions* in which increased protein katabolism and urinary nitrogen excretion are observed.

TABLE 21

Blood regeneration—sugar and gliadin—metabolism. Dog 16–160. White bull mongrel, female, young adult

DATE, 1917	PIGMENT VOLUME = Hb PERCENTUM × BLOOD VOLUME	BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	R. B. C. HEMATOCRIT	Hb	COLOR INDEX	R. B. C. MILLION	W. B. C.	WEIGHT	BLOOD PER KILOGRAM	REMARKS
		cc.	*cc.*	*cc.*	*per cent*	*per cent*				*kgm.*	*cc.*	
10/17	1397	1083	509	627	53.0	118	0.74	8,0	8,2	8.90	122	
10/17	Diet: Bread and milk											
10/19	Bled 271 cc.											
10/20	Bled 271 cc.											
10/22	600	844	574	270	32.0	71	1.04	3,4	11,4	8.90	95	
10/22	Diet: 75 grams cane sugar, 25 grams glucose, 300 cc. water											
10/29	617	812	552	260	32.0	76	0.88	4,3	8,2	8.0	101	*
11/5	770	794	548	246	31.0	97	0.87	5,6	6,0	7.4	105	*
11/5	Diet: 75 grams cane sugar, 25 grams glucose, 20 grams gliadin, 300 cc. water											
11/12	762	838	536	302	36.0	91	0.78	5,8	3,6	7.10	118	*
11/18	764	813	496	317	39.0	94	0.82	5,7	7,2	6.70	121	*
11/26	780	777	466	311	40.0	103	0.76	6,6	4,4	6.40	106	*
12/3	755	770	485	285	37.0	98	0.71	6,9	3,6	6.30	106	*

* Poikilocytosis and anisocytosis of red cells.

Blood volume with dry oxalate. Hemoglobin by Sahli tubes.

Gliadin prepared in the usual way by dilute alcohol extraction of wheat flour.

Experimental history, see table 18-b.

Tables 21 and 21-a give more data on sugar feeding periods. The hemoglobin regeneration is rather more marked than in the average experiment during a 2-week period. We note again a remarkable increase in red cells (2,000,000) and hemoglobin (26 per cent) with a stationary red cell hematocrit (32 per cent). If this observation had not been recorded in other experiments we might suspect an error.

TABLE 21-A

Total urinary nitrogen—sugar and gliadin. Dog 16–190

DATE, 1917	TOTAL NITROGEN 24 HOURS URINE	URINE TOTAL 24 HOURS	WEIGHT	REMARKS
October 22	Diet: 75 grams cane sugar, 25 grams glucose, 300 cc. water daily			
	grams	*cc.*	*pounds*	
23	2.77	413	18.6	0 feces
24	2.02	267	18.3	Solid feces
25	1.74	232	18.2	0 feces
26	1.46	178	17.8	Trace of feces
27	1.48	101	17.8	0 feces
28	1.62	251	17.8	0 feces
29	1.74	261	17.6	0 feces
30	2.10	256	17.3	Diarrhea +
31	1.43	217	17.5	0 feces. Vomited
November 1	1.51	131	16.8	0 feces
2	1.57	241	16.8	0 feces
3	1.46	471	16.0	0 feces
4	1.79	151	16.3	0 feces
November 5	Diet: 75 grams cane sugar, 25 grams glucose, 20 grams gliadin, 300 cc. water daily			
5	1.71	301	16.2	Trace feces
6	3.16	241	16.1	0 feces
7	3.53	192	15.9	Feces +
8	3.47	186	15.9	Diarrhea +
9	3.25	173	15.8	Diarrhea +
10	3.58	311	15.5	Diarrhea ++
11	3.25	277	15.4	0 feces
12	3.33	322	15.6	0 feces
13	3.47	301	15.3	Solid feces
14	3.53	319	15.1	Diarrhea +
15	3.50	156	15.1	Trace of feces
16	3.30	132	15.0	0 feces
17	3.25	176	14.9	Soft feces
18	3.30	151	14.7	Trace of feces
19	3.36	192	14.8	Diarrhea +
20	3.25	161	14.6	0 feces
21	3.36	156	14.5	Soft feces
22	3.22	151	14.4	Solid feces
23	3.58	156	14.4	Soft feces
24	3.47	182	14.3	Soft feces
25	3.36	181	14.2	Soft feces
26	3.33	173	14.1	Diarrhea +
27	3.47	222	13.9	Soft feces
28	3.22	152	14.0	Trace of feces
29	3.36	181	13.9	0 feces
30	3.08	168	13.9	Soft feces
December 1	3.70	166	13.9	0 feces
2	3.28	181	13.8	Trace of feces
3	3.42	163	13.8	0 feces

TABLE 22

Blood regeneration—sugar plus carrot extract—yeast, bread and milk. Dog 16–166. White mongrel, female, adult

DATE, 1919	PIGMENT VOLUME = Hb. PER CENT TIMES BLOOD VOLUME	BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	R. B. C. HEMATOCRIT	Hb.	COLOR INDEX	R. B. C. MILLION	W. B. C.	WEIGHT	BLOOD PER KILOGRAM	REMARKS
		cc.	*cc.*	*cc.*	*per cent*	*per cent*				*kgm.*	*cc.*	
2/20	1375	1114	508	601	53.9	123	0.70	8.8	6.0	11.30	99	
2/20	Diet: Bread and milk											
2/21	Bled 280 cc.											
2/22	Bled 280 cc.											
2/24	690	884	473	307	34.7	78	0.85	4.6	19.2	10.45	85	
2/21	Diet: 100 grams sugar, 100 cc. carrot juice,† 150 cc. water											
2/27	548	816	569	242	29.7	67	0.90	3.7	12.8	10.10	81	
3/5	500	818	568	238	29.1	61	0.71	4.3	11.6	9.65	85	
3/12	444	776	555	206	26.5	57	0.73	3.9	5.2	9.15	85	* Poik.++
3/18	474	750	530	211	28.2	63	0.79	4.0	6.8	8.75	86	* Poik.++ Shadow cells
3/18	Diet: 200 grams bread, 500 cc. milk											
3/26	520	812	575	232	28.6	64	0.71	4.5	7.4	9.20	88	* Poik.++
3/26	Diet: 200 grams bread, 3 grams yeast, 300 cc. milk											
4/2	516	816	556	251	30.8	63	0.63	5.0	6.2	9.45	86	* Poik.++
4/8	433	761	542	211	27.7	57	0.54	5.3	9.4	9.35	81	* Poik.++
4/14	416	726	517	197	27.5	57	0.57	5.0	7.2	9.30	78	* Poik.
4/21	535	828	553	275	31.2	65	0.59	5.5	9.4	9.40	88	
4/21	Diet: 200 grams cooked beef liver, 200 grams bread, 300 cc. milk											
4/28	631	851	542	305	35.8	74	0.70	5.3	20.4	10.30	83	R. B. C. fairly normal
5/7	1087	1055	548	496	47.0	103	0.71	7.3	10.4	10.70	99	
5/12	1153	1082	556	517	47.6	107	0.69	7.8	16.2	10.85	100	

* Poikilocytosis of red cells.

† Carrot juice = water extract of cooked carrots filtered and concentrated to one-third of its original volume.

Experimental history, see table 18–b.

This shows the possible fluctuation in hemoglobin content and size of red corpuscles which may be observed with a constant hematocrit. Probably many factors enter into this reaction which will be taken up again. The clinical summary of this dog shows a variety of diet periods including a fasting period (table 18-a).

Gliadin (20 grams per day) added to the sugar diet causes no increase in hemoglobin but merely a uniform maintenance factor. There is still further increase in red cells with poikilocytosis and we believe the evidence favors some red cell fragmentation under these conditions. The urinary nitrogen shows a uniformly low level during the sugar period and the expected level during gliadin feeding (table 21-a).

Table 22 gives the results of a second sugar regeneration period on the same dog (16-160) used in the preceding experiment (table 21). It will be observed that this sugar regeneration period is not as favorable and there is actually a loss in pigment volume amounting to about 30 per cent during a period of 4 weeks. This experiment includes another factor (carrot juice) which is obviously inert under these experimental conditions. This point will come up again in subsequent papers dealing with pigment derivatives. We wish to point out an unusual condition on February 24 after the 2 bleeding days when a low plasma volume (473 cc.) is recorded against the normal during the entire experiment of 500 to 550 cc. When the plasma volume returns to normal we note a fall in hemoglobin and red cell hematocrit. It is probable that the reaction is the result of the shock of the hemorrhage which is usually adjusted during the resting day intervening between the second bleeding and the second blood volume determination.

The second period of bread and milk feeding is included to substantiate the plasma volume figures. This reaction will be discussed in detail in the next paper.

Table 23 is given at the end of the series because one important factor separates it from all the other experiments,—*a bile fistula*. This dog (15-22) has been under observation in this laboratory for several years and many reports on bile excretion include experiments on this animal (4). It is known that traces of bile can gain entrance into this dog's intestine but the general condition of the dog is perfect. The reaction to anemia under fasting conditions in this dog is of particular interest as the absorption of pigments from the intestine may be excluded. It has been suggested by Addis (5) that absorption of some pigment complex from the intestine is a part of the body conservation of pigment materials. This and other experiments give no support to this interesting suggestion.

Bile pigment figures in a fasting period are given for this same dog in another publication (6). The constant presence of *urobilin* in the fasting bile of this experiment is noted in table 23 and discussed below. Total urinary nitrogen figures are not given here but were obtained in this experiment and average 3.08 grams per 24 hours. This is an average figure for a normal dog of similar weight and activity. Evidently

TABLE 23

Blood regeneration—fasting—metabolism—bile fistula. Dog 15–22. Brindle bull, male, age 4 years +

DATE, 1917	PIGMENT VOLUME = Hb. PER CENT TIMES BLOOD VOLUME	BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	R. B. C. HEMATOCRIT	Hb.	COLOR INDEX	R. B. C. MILLION	W. B. C.	WEIGHT	BLOOD PER KILOGRAM
		cc.	*cc.*	*cc.*	*per cent*	*per cent*				*kgm.*	*cc.*
3/26	2200	1833	770	1063	58.0	120	0.77	7.8	12.6	15.40	119
3/26	Diet: Bread and milk										
3/28	Bled 460 cc.										
3/29	Bled 460 cc.										
3/30	673	962	654	308	32.0	70	0.83	4.2	18.6	15.10	63
3/30	Fasting begun										
4/2	890	1186	747	439	37.0	75	0.66	5.7	28.2	14.00	85
4/9	874	1136	625	511	45.0	77	0.70	5.5	14.6	12.50	91
4/16	1122	1069	556	513	48.0	105	0.71	6.4	6.2	11.40	94
4/23	1133	1059	540	519	49.0	107	0.66	8.1	7.2	10.40	116

Blood volume with dry oxalate. Hemoglobin by Sahli tubes.
No previous anemia experiments on this dog.
Urobilin constantly present in bile after 1st day of fasting period.
Total nitrogen in urine—average 3.08 grams per 24 hours.

the bile fistula does not modify the total nitrogen figures in this experiment nor in many others.

The curve of hemoglobin and pigment volume is quite remarkable. There is even more marked a new formation of red cells and hemoglobin than is noted in an average normal dog. We must not forget the occasional abnormal regeneration which is noted in a normal dog which has never been bled—a reserve being forthcoming which returns the

hemoglobin almost to normal in 3 weeks of fasting. This bile fistula dog had never been bled in any large amounts before. But the reserve reaction in this dog is truly remarkable and at the end of the fasting period we note a normal red count, a hemoglobin and red cell hematocrit figure close to normal and a considerable increase in pigment volume. It is noted that the plasma volume shows a decrease during the fasting period which gives the reason for the relatively low pigment volume. At any rate the capacity of this bile fistula dog for hemoglobin regeneration is in no way impaired and *may* be greater than normal.

TABLE 24

Fasting summary

DOG NUMBER	AFTER BLEEDING			TWO WEEKS LATER			THREE WEEKS LATER			FOUR WEEKS LATER		
	Pigment volume	Hematocrit R. B. C.	Hemoglobin	Pigment volume	Hematocrit R. B. C.	Hemoglobin	Pigment volume	Hematocrit R. B. C.	Hemoglobin	Pigment volume	Hematocrit R. B. C.	Hemoglobin
15–22	673	32.0	70	874	45.0	77	1122	48.0	105	1133	49.0	107
18–103	590	25.3	62	852	36.4	97	745	35.5	96	700	37.7	89
18–114	620	30.1	62	899	38.7	93	1103	44.4	102			
18–116	778	28.5	62	1025	36.0	85	1176	40.7	95			
17–38	443	28.0	60	491	39.0	68	585	43.0	76	585	44.0	78
17–37	313	28.0	50	492	35.0	64	532	35.0	73	540	35.0	70
16–160	482	33.2	64	591	33.5	88	636	34.3	90			
17–28	631	30.8	66	1007	43.8	102						
17–27	1005	31.0	90	1086	39.0	101	1213	41.0	115	1240	38.0	124
Fasting average.....	615	29.65	65	813	38.5	86	889	40.23	94	840	40.74	94
Sugar average, table 25	515	30.87	63	648	35.5	75	593	36.4	76	614	37.04	74

For example, we know that the bile fistula liver contains more pigment as demonstrated by the microscope. There may be stored away in the liver or other tissues more of the pigment complex from which the pigment reserve is derived—to be turned into finished hemoglobin on emergency demand.

The last three tables (tables 24, 25 and 25-a) give the summary figures of all the experiments in this paper and one fact stands out clearly in table 25-a. The *average blood regeneration* is distinctly greater during a fasting period of 2 weeks than during a similar sugar period of 2

weeks. The difference is even more striking at the end of 3 weeks when the average gain in pigment volume during fasting is 274, contrasted with 78 on sugar feeding. Hemoglobin figures show an average gain of 29 per cent on fasting contrasted with 13 per cent on sugar diet.

TABLE 25

Sugar diet summary

DOG NUMBER	AFTER BLEEDING			TWO WEEKS LATER			THREE WEEKS LATER			FOUR WEEKS LATER		
	Pigment volume	Hematocrit R.B.C.	Hemoglobin	Pigment volume	Hematocrit R.B.C.	Hemoglobin	Pigment volume	Hematocrit R.B.C.	Hemoglobin	Pigment volume	Hematocrit R.B.C.	Hemoglobin
17–28	588	34.0	61	636	38.0	62	634	39.0	66	541	40.0	58
17–34	414	30.0	53	536	32.0	60	497	32.0	56	556	35.0	64
17–38	468	30.0	53	574	38.0	76	668	41.0	84	735	43.0	89
16–160	600	32.0	71	770	31.0	97	762	36.0	91	764	39.0	94
16–160	690	34.7	78	500	29.1	61	444	26.5	57	474	28.2	63
19–28	316	26.9	62	916	47.2	107	562	47.3	121			
17–27	598	34.0	63	763	41.0	70	750	42.0	73			
16–160	448	25.5	64	488	27.3	69	429	27.4	64			
Sugar average	515	30.87	63	648	35.5	75	593	36.4	76	614	37.04	74
Fasting average, table 24	615	29.65	65	813	38.5	86	889	40.23	94	840	40.74	94

TABLE 25-A

Gains made above minimum level after bleeding

	DURING TWO WEEKS (TOTAL)			DURING THREE WEEKS (TOTAL)		
	Pigment volume	Hematocrit R.B.C.	Hemoglobin	Pigment volume	Hematocrit R.B.C.	Hemoglobin
Nine experiments, fasting average	198	8.85	21	274	10.58	29
Eight experiments, sugar average	133	4.63	12	78	5.53	13

DISCUSSION

The term "sparing action of carbohydrates" is familiar to all workers in metabolism and means that carbohydrate feeding will decrease the excretion of total urinary nitrogen by man or animal as compared with the fasting excretion of nitrogen. It is therefore assumed that

the administration of carbohydrate actually spares the body protein. There are two theories for this sparing action of the carbohydrates: *a*, That the sparing action is at the source—that is, the sugar prevents tissue katabolism or spares protein in tissue cells; *b*, That the sparing action is a conservation of split products which, with the aid of carbohydrate radicles, are reconstructed into a variety of protein complexes and used in various parts of the body. Both theories have able advocates, but it is fair to say that most of the experiments can be used by a skilful proponent to support either hypothesis. An admirable review of the work in this field has been published recently by Janney (7).

Some experiments by Davis and Whipple (8) concerning the regeneration of liver cells following a unit injury can be said to support convincingly the theory of conservation of protein end products. In these experiments there is little liver cell repair during fasting periods but a rapid repair during sugar diet periods. This indicates that the body can form many new liver cells when sugar is available but not during a fasting period. These new liver cells must be formed from body protein split products, and this type of conservation of nitrogen is due to sugar feeding and cannot be explained by any amount of protein sparing at the source.

The experiments, summarized in tables 24, 25 and 25-a, follow the normal regeneration of another type of body cell: the normal red blood cell which is constantly being used up and reformed or reconstructed day by day. This is an admirable cell for a study of cell regeneration as its main constituent (hemoglobin) is very complex and easily and accurately measured. The amount of hemoglobin circulating in the body can be measured with considerable accuracy and therefore its curve of regeneration can be established with reasonable precision. When we compare periods of regeneration of hemoglobin during fasting and during sugar diet periods we find a constant difference which comes out clearly in an average figure of many experiments (table 25-a). This table shows that the fasting dog can regenerate more red cells and hemoglobin than a dog on a sugar diet. This figure is over and above the maintenance supply of hemoglobin which is needed to keep the level uniform and furnish the hemoglobin used up by the daily wear and tear of the circulation in the body.

This actual reconstruction of new red cells and hemoglobin must come from the body protein or its split products as no nitrogen is being supplied to the body. Evidently the body conserves very carefully the substances which are suitable for the elaboration of hemoglobin. The

pyrrol complex is a peculiar feature of the hemoglobin molecule which can scarcely be formed in the body and may be an important determining factor in its reconstruction. How may we explain the increase in hemoglobin during fasting periods in excess of the reaction on sugar feeding? This surely cannot be explained by increased synthetic capacity due to the presence of sugar or the conditions should be reversed. If we assume that sugar may have a certain sparing action at the source we are able to suggest a plausible explanation. Suppose the sugar feeding does protect body protein from katabolism and therefore lessens the amount of available protein split products, we are then able to explain the smaller amount of hemoglobin produced, *provided* we assume that under all circumstances of need or limited diet the body conserves *all* the available protein building stones which go to make up the hemoglobin molecule. This seems to us to be the best explanation of the observed facts, but this opinion may be modified by further work.

It may be objected that the "maintenance factor" of red cells may vary in fasting as compared with sugar periods. This factor is not to be determined at this time, but we have no reason to suppose that the daily wastage of red cells should be greater on sugar feeding than during fasting periods. This question must be left open for the present.

Granting the facts as outlined above we may say that we have good proof to explain the "sparing action of carbohydrates" as due to a conservation of protein split products which aid in new protein construction as observed in liver repair (8). But these anemia experiments may be best explained by a "sparing action of carbohydrate" which protects at the source the body protein from katabolism. If this work is correct we may assume that the carbohydrate in the diet may have a double "sparing action"—*to protect the body protein at its source and to aid materially in the conservation of protein split products*, which are recast into new body protein. That one or the other of these two reactions may be dominant under varying conditions may be granted as probable.

No discussion of any phase of pigment metabolism is complete without proper consideration of the pigment *output in the bile*. The bile pigment represents in part at least under certain conditions the end product of hemoglobin degradation in the body, but in addition under certain conditions this bile pigment may represent certain constructive activities of the liver (9). It is possible that a part of this bile pigment produced by the liver and not derived from the degradation of hemoglobin may be an excess of pigment substance available for hemoglobin *production* but not so used and later discarded by way of the bile. This

reaction might only take place in the liver when there was a considerable surplus of pigment elements in the food or elsewhere. Such conditions might not obtain in the body when it was deprived of all protein intake (fasting or sugar diet).

The bile pigment output, therefore, must be reviewed briefly in this place. We are able to refer to observations of this nature (6) and state that there may or may not be differences in the bile pigment excretion during fasting periods as compared with sugar feeding periods in bile fistula dogs. Certain experiments appear to show greater bile pigment elimination during sugar periods than during fasting periods but other experiments show little or no difference in the bile pigment figures. These differences may represent individual variations in these bile fistula dogs and the available data are not sufficient to establish any difference in bile pigment excretion under these conditions. One thing is quite clear—these bile fistula dogs during fasting or sugar periods do excrete a measurable amount of bile pigments (average of 15 to 30 mgm. bile pigment per 6 hour daily collection). This pigment results from the degradation of hemoglobin in the body or the production of pigment complex from other substances in the liver—in other words, a distinct loss of pigment material from the body.

One other point must be mentioned in this connection. During fasting periods in bile fistula dogs we have noted the invariable appearance of *urobilin in the bile*. In our experience this is the only condition which is constantly associated with urobilin production in the dog's liver. At times the bile pigments may be almost completely replaced by the urobilin pigment and this introduces a serious error in our analysis of bile pigment. It is probable (if not certain) that this urobilin is derived from the bile pigment and its increase therefore will be associated with a corresponding decrease in bile pigments. But we have no accurate quantitative analytical method for urobilin, although we can estimate bile pigments there present by precipitation of the calcium pigment compound, filtration and analysis of the acid alcohol derivative. It seems probable to us that the urobilin appearing in the bile fistula dogs is derived at least in part from the bile pigments in the bile ducts, due to the activity of bacteria which we know are responsible in part at least for this reaction in the intestine. During fasting periods the flow of bile is very sluggish and this inferior drainage of bile gives a favorable opportunity for the bacteria to multiply. It can be stated that bacteria are numerous in all bile fistula tracts and may at times set up an inflammatory reaction in the bile passages which will cause

trouble. Flushing out the bile passages by cholagogue action as a rule gives relief to this condition.

It has occurred to us that this observation may have some significance as regards urobilin in the urine in a variety of conditions. There is no conclusive proof that urobilin is ever absorbed from the intestine. We have here proof that urobilin may be formed in the liver. It would seem safe to assume that the *hepatic origin of urobilin* should be considered in any analysis of this complex question. When the possibility is suggested that *urobilin may be formed at times in the liver*, it is obvious how difficult it is to exclude this *possibility* in the clinical conditions associated with which we note urobilin in the urine.

SUMMARY

During *fasting periods* after unit hemorrhages the normal dog can regenerate measurable amounts of red cells and hemoglobin.

This regeneration of red cells and hemoglobin *includes* the daily wastage of these elements, or the maintenance factor of the blood. The curve of hemoglobin regeneration represents the production of hemoglobin *in excess* of this unknown maintenance factor.

Bile pigment excretion under fasting or sugar diet conditions may be considered as uniform. A bile fistula dog may regenerate hemoglobin and red cells with at least equal and perhaps greater speed than a normal dog. The constant presence of *urobilin* in the bile of the fasting bile fistula dog is recorded and discussed from the standpoint of *urobilinuria*. The hepatic origin of urobilin is suggested.

During *sugar diet periods* the regeneration of hemoglobin and red cells is *distinctly less than during fasting periods* (table 25-a).

We believe that this observation may be explained by a double "sparing action of carbohydrates"—both sparing at the source or protecting body protein from katabolism as well as effecting synthetically a distinct conservation of protein split products. This postulates a strict conservation by the body of certain protein fractions which may be recast into hemoglobin. The presence of carbohydrate may facilitate this reaction but the actual new formation of hemoglobin may depend in part upon the type and amount of amino acid groups available from normal protein katabolism.

Histidine given with sugar appears to cause a production of hemoglobin over the control level. This amino acid may be one of the important elements in this hemoglobin regeneration complex.

Gliadin in the amounts used does not modify the hemoglobin reaction.

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BLOOD REGENERATION FOLLOWING SIMPLE ANEMIA

III. Influence of Bread and Milk, Crackermeal, Rice and Potato, Casein and Gliadin in Varying Amounts and Combinations

C. W. HOOPER, F. S. ROBSCHEIT and G. H. WHIPPLE

From the George Williams Hooper Foundation for Medical Research, University of California Medical School, San Francisco

Received for publication April 3, 1920

Early in our work it became evident that bread and milk did not constitute a favorable diet for the rapid regeneration of blood. This diet is palatable to dogs and maintains them in good nutritional condition for many weeks. As a result we began to use this diet as a maintenance diet to which other factors could be added which did or did not modify the curve of blood regeneration to be expected from the bread and milk factors alone. Because in many experiments we use bread and milk as a part of the diet it is essential that we understand clearly the effect of this diet under a variety of conditions. We therefore submit many experiments tabulated below to establish the normal blood regeneration of the dog which is limited to varying amounts of dried white bread and skim milk.

We must mention in passing the dietary deficiency disease which develops in dogs kept for long periods on a strict bread and milk diet. This disease condition resembles scurvy in human beings and is rapidly fatal if not energetically treated by antiscorbutic measures. This condition will be reviewed in a subsequent publication.

It will be noted from the experiments here outlined that bread and milk alone when given in large amounts may return the blood picture to normal in six weeks or longer. But when given in moderate amounts (100 grams dried bread and 500 cc. skim milk) this diet will rarely permit of complete blood regeneration. On this diet the hemoglobin, pigment volume and red cell hematocrit may be kept at a permanently subnormal level following the unit hemorrhages used in our experiments to produce uncomplicated secondary anemia. The value of

establishing this fact is obvious when it is found that some food materials added to this diet will profoundly modify this reaction expected from bread and milk alone. These observations will be reported in subsequent communications.

A few experiments with two of the important constituents of the bread and milk diet are included. Casein and gliadin in sufficient amounts are able to modify somewhat the reaction expected from sugar feeding alone. Some work with incomplete proteins and mixtures of amino acids has been completed but the evidence, so far, is not conclusively in favor of any single amino acid as being responsible for this peculiar reaction which depends in great measure upon the capacity of the body to construct hemoglobin.

Crackermeal and milk were used at one period during the war when white bread or in fact any kind of bread was not available for obvious reasons. This crackermeal was purchased on the open market during the war period and its constitution is not accurately known. We were able to ascertain with reasonable certainty that this crackermeal contained 70 to 80 per cent wheat flour, but a considerable percentage of barley and rice flour. Other grains may possibly have been concerned. The fact remains that this mixture of wheat flour and other grain flours did not modify in any manner the reaction established recently for commercial white bread which at present is made almost wholly from wheat flour.

Rice, potatoes and milk are used in one large series of experiments. The amount of blood regeneration on this diet closely parallels that observed on a bread and milk diet. This diet also includes one other substance sometimes used in bread,—potato or potato flour. Any one of these three diets is a favorable maintenance diet to which other factors may be added to determine the value of the unknown substance in its relation to blood regeneration.

EXPERIMENTAL OBSERVATIONS

Unless otherwise noted, the same technique is used in these experiments which has been described above (paper I). The food mixtures were all palatable and readily eaten unless note is made to the contrary. With few exceptions the dogs maintained their weight, general activity and health throughout the experiments.

Bread and milk diet. The first experiment (table 26) in this series illustrates many points which are established by the succeeding experi-

ments given below. The dog presented a very high hemoglobin (130 per cent), high red count (10 million) and blood per kilo (127 to 117 cc.). Following the unit hemorrhages there is recorded as usual a volume of red cells (239 cc.) which is much below the calculated expected red cell volume (438 cc.). The low level in this experiment is more noticeable than in the average experiment. The plasma volume is promptly made up to normal after the bleedings and remains as usual relatively constant. The curve of regeneration is quite steep during the first 2 weeks of the bread and milk diet but thereafter remains at a uniform level. This statement applies to the pigment volume, red cell hematocrit, and hemoglobin, but the red cell count shows a slow increase toward normal in the last 3 weeks of the experiment.

In this first experiment the diet was abundant and sufficient to maintain the body weight and even to allow of slight increase. This point is of much importance, as will appear later. This and other similar experiments show that a *liberal bread and milk diet* sufficient to maintain or increase the body weight will cause a certain degree of hemoglobin regeneration over and above the daily maintenance hemoglobin factor. Blood regeneration may be rapid for a week or two and may even return the blood picture almost to normal in certain experiments.

The repeat experiment on this same dog (table 26) shows a lower initial hemoglobin and red count. The hemorrhages are less in amount but the anemia level for pigment volume is much the same as in the preceding anemia period. The diet now is not abundant and contains only 100 grams dried bread as compared with 343 grams in the first period of regeneration. This 100 grams bread diet is not sufficient to maintain the body weight at its normal level and there is a loss in weight of 1.4 kilos during the 5 weeks of blood regeneration. There is a striking difference in the amount of hemoglobin regeneration which shows only a trivial increase from week to week over the maintenance factor. In using the term *maintenance factor* we wish to indicate that unknown replacement fraction which represents the daily wastage of red cells used up in the body metabolism. There are experiments to indicate that this fraction may be 3 per cent per day in human beings, but there are no data to establish this important point for the dog.

It is obvious from these two anemia periods (table 26) that a dog will regenerate more hemoglobin on an abundant bread and milk diet than on a limited bread and milk diet. This applies particularly to the *bread* portion of the diet. The term "bread" as used in this

TABLE 26

Blood regeneration—bread and milk—repeat experiment. Dog 17–38. Bull mongrel, female, young adult

DATE, 1919	PIGMENT VOLUME = Hb. PER CENT TIMES BLOOD VOLUME	BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	R. B. C. HEMATOCRIT	Hb.	COLOR INDEX	R. B. C. MILLION	W. B. C.	WEIGHT	BLOOD PER KILOGRAM	REMARKS
		cc.	*cc.*	*cc.*	*per cent*	*per cent*				*kgm.*	*cc.*	
1/30	1910	1467	585	877	59.8	130				11.5	127	
2/6	1873	1418	600	812	57.2	132	0.66	10.0	9,0	12.15	117	
1/30	Diet: White bread and milk											
2/7	Bled 355 cc.											
2/8	Bled 355 cc.											
2/10	520	852	608	239	28.1	61	1.0	3,1	28,4	11.1	77	
2/10	Diet: Dried, ground white bread, 343 grams, skim milk, 500 cc.											
2/17	852	1074	654	404	37.6	79	0.88	4,5	12,0	11.6	93	
2/17	Diet: Dried, ground white bread, 343 grams, skim milk, 200 cc.											
2/26	1120	1133	588	534	47.1	99	0.72	6,9	11,8	11.6	98	
3/3	1137	1110	560	544	49.0	102	0.82	6,2	7,4	11.35	98	* Poik. +
3/10	1138	1083	575	497	45.9	105	0.72	7,3	7,8	11.5	94	* Poik. +
3/19	1092	1072	561	493	46.0	102	0.66	7,7	6,8	11.35	95	* Poik. +
3/21	Mixed diet											
3/31	1275	1155	558	586	50.7	110	0.67	8,2	7,2	12.0	96	* Poik. ++
3/31	Diet: White bread and milk											
4/1	Bled 290 cc.											
4/2	Bled 290 cc. No distress											
4/3	499	887	633	249	28.1	56	0.67	4,2	9,6	11.4	78	* Poik. +
4/3	Diet: Dried, ground white bread, 100 grams, skim milk, 500 cc.											
4/11	566	989	662	307	31.0	64	0.68	4,7	11,0	11.25	77	*
4/18	662	988	638	335	33.9	67	0.56	6,0	9,2	11.0	90	*
4/25	652	920	578	304	35.4	71	0.55	6,5	6,6	10.7	86	*
5/2	734	955	585	367	38.4	77	0.57	6,7	7,2	10.35	92	* Poik. ++
5/9	691	909	564	341	37.5	76	0.58	6,5	5,6	10.0	91	* Poik. ++

* Poikilocytosis of red cells.

Experimental history, see table 20–b.

TABLE 27

Blood regeneration—bread and milk—repeat experiment. Dog 19–94. Bull mongrel, male, age 5 months

DATE, 1919	PIGMENT VOLUME = Hb. PER CENT TIMES BLOOD VOLUME	BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	R. B. C. HEMATOCRIT	Hb.	COLOR INDEX	R. B. C. MILLION	W. B. C.	WEIGHT	BLOOD PER KILOGRAM	REMARKS
		cc.	*cc.*	*cc.*	*per cent*	*per cent*				*kgm.*	*cc.*	
1/16	1250	1343	790	540	40.2	93	0.57	8.1	21,6	10.65	126	
1/16	Diet: Bread and milk											
1/17	Bled 335 cc.											
1/18	Bled 285 cc. No distress											
1/20	462	839	645	190	22.6	55	0.95	2,9	14,6	10.00	84	
1/20	Diet: 250 grams dried, ground white bread, 500 cc. skim milk											
1/27	576	1020	683	327	32.0	56	0.55	5,1	11,0	10.25	100	*
2/3	900	1088	651	485	39.7	83	0.57	7,3	13,4	10.25	106	* Poik. +
2/12	750	1000	626	362	36.2	75	0.72	5,2	10,2	10.80	93	* Poik. +
2/19	907	1085	647	439	40.4	84	0.69	6,1	9,8	11.15	97	*
2/28	994	1126	667	448	39.8	88	0.76	5,8	6,2	11.50	98	*
3/7	899	1098	650	437	39.8	82	0.73	5,6	8,4	11.70	94	*
3/10	Diet: Mixed diet											
3/17	1146	1216	687	523	43.0	94	0.70	6,7	10,6	13.05	93	* Slight
3/17	Diet: White bread and milk											
3/18	Bled 304 cc.											
3/19	Bled 304 cc. No distress											
3/21	542	968	709	255	26.3	56	0.82	3,4	14,4	12.40	78	
3/21	Diet: 300 grams dried, ground white bread, 500 cc. skim milk											
3/28	736	979	640	324	33.1	75	0.89	4,2	12,6	12.50	94	* Slight
4/2	811	1112	712	395	35.5	73	0.78	4,7	8,4	12.45	89	*
4/9	864	1110	680	420	37.8	78	0.65	5,9	11,4	12.40	89	* Slight
4/16	1005	1200	718	469	39.1	84	0.70	6,0	8,0	12.75	94	* Slight
4/23	1094	1236	740	485	39.2	88	0.71	6,9	6,0	12.75	97	* Slight
4/30	1031	1311	800	498	38.0	79	0.55	7,2	10,0	13.20	99	* Slight

* Poikilocytosis of red cells.

No previous anemia experiments with this dog.

report indicates white bread of the first quality, obtained from the University Hospital, sorted, dried in an oven and pulverized.

Table 27 gives a repeat experiment which shows that the curve of blood regeneration following a very short resting period is practically identical under uniform diet conditions. It is of considerable importance to know beyond question whether we may expect a uniform reaction under uniform conditions when a dog is used for different experiments at different intervals of time. We believe this communication includes sufficient data to establish this point beyond question. Therefore we may feel secure in using for anemia work the same set of dogs, *provided* the blood picture has returned to normal and the weight and general health is also normal. With repeated anemia experiments the dog does not increase in its capacity to regenerate hemoglobin nor does this reparative mechanism fail under the conditions of these repeat experiments. We may then attach considerable significance to deviations from the standard reaction in any given animal.

The repeat experiment (table 27) gives a reaction curve of pigment volume, red cell hematocrit and hemoglobin which is practically identical with the first anemia period. There is slightly more gain in the repeat experiment than in the first anemia observation. In both anemia periods the diet was liberal and permitted a gain in body weight of approximately 1 kilo per 6-week period. The repeat experiment brought the hemoglobin back more nearly to normal but the initial anemia level was not as low nor was the loss by hemorrhage as great. In the repeat experiment the dog received 300 grams dried white bread in contrast to 250 grams bread in the first period, but this was only a proper proportion per kilo body weight. These dogs, moreover, were in a period of rapid body growth (5 to 8 months).

The anemia experiments given in table 28 are very similar to those just described. In this instance, too, the bread and milk diet was sufficient for maintenance plus a definite growth factor with a gain of 2 kilos in body weight during the 5-week periods. In both periods the regeneration brings the pigment volume and blood picture back to the normal level. The steady gain in weight and body growth must not be lost sight of in reviewing the same gain in plasma volume. In the adult normal dog the plasma volume is now known to be quite constant under these experimental conditions.

The experiment given in table 29 is slightly different from those preceding. After the anemia period we have 1 week's fast which shows as usual a definite gain in pigment volume. The subsequent 3 weeks

TABLE 28

Blood regeneration—bread and milk—repeat experiment. Dog 19–102. Bull mongrel, male, age 5 to 6 months

DATE, 1919	PIGMENT VOLUME = Hb. PER CENT TIMES BLOOD VOLUME	BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	R. B. C. HEMATOCRIT	Hb.	COLOR INDEX	R. B. C. MILLION	W. B. C.	WEIGHT	BLOOD PER KILOGRAM	REMARKS
		cc.	*cc.*	*cc.*	*per cent*	*per cent*				*kgm.*	*cc.*	
1/30	972	944	482	453	48.0	103				9.75	97	
2/6	1026	1068	606	452	42.3	96	0.63	7,6	16.0	10.90	98	
2/6	Diet: Bread and milk											
2/7	Bled 267 cc.											
2/8	Bled 267 cc. No distress											
2/10	418	803	594	205	25.5	52	0.79	3,3	11,4	10.25	78	
2/10	Diet: 283 grams dried, ground white bread, 500 cc. skim milk											
2/17	720	951	608	337	35.5	76	0.93	4,1	8,6	10.65	89	
2/26	888	1014	592	417	41.1	87	0.65	6,7	11,6	11.25	90	* Poik. +
3/3	1045	1068	575	488	45.7	98	0.76	6,4	12,8	11.35	94	* Poik. +
3/10	1020	1045	578	462	44.2	97	0.73	6.6	11,2	11.55	91	* Poik. ++
3/19	1026	1056	576	467	44.2	97	0.70	6,9	12,0	12.55	86	* Poik. ++
3/22	Diet: Mixed diet											
3/31	1220	1220	644	564	46.2	100	0.67	7,5	12,6	13.20	93	* Poik. ++
3/31	Diet: Bread and milk											
4/1	Bled 305 cc.											
4/2	Bled 305 cc. No distress											
4/3	470	874	634	232	26.5	54	0.68	4,0	16,8	12.50	70	* Poik. ++
4/3	Diet: 283 grams dried, ground white bread, 500 cc. skim milk											
4/11	758	1085	702	361	33.3	70	0.69	5,1	7,4	13.30	82	*
4/18	884	1083	650	421	38.9	82	0.64	6,4	12,8	13.50	80	
4/25	1099	1163	660	498	42.8	94	0.64	7,3	9,6	13.85	84	*
5/2	1648	1485	701	769	51.8	111	0.74	7,5	11,2	14.55	102	*
5/9	1280	1268	667	584	46.0	101	0.64	7,9	19,8	14.50	87	*

* Poikilocytosis of red cells.

No previous anemia experiments with this dog.

show a stationary pigment volume up to the last week, when there is a definite gain. This diet was sufficient for maintenance of body weight and the general condition was uniformly excellent.

TABLE 29

Blood regeneration—bread and milk. Dog 18–123. Brindle bull mongrel, male, young adult

DATE, 1919	PIGMENT VOLUME: Hb. PER CENT TIMES BLOOD VOLUME	BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	R. B. C. HEMATOCRIT	Hb.	COLOR INDEX	R. B. C. MILLION	W. B. C.	WEIGHT	BLOOD PER KILOGRAM	REMARKS
		cc.	*cc.*	*cc.*	*per cent*	*per cent*				*kgm.*	*cc.*	
5/1	1692	1270	546	716	56.9	133	0.68	9,8	12,2	12.55	101	
5/1	Diet: Bread and milk											
5/2	Bled 318 cc.											
5/3	Bled 318 cc. No distress											
5/5	592	826	552	270	32.7	72	0.64	5,6	18,8	11.60	71	
5/6	Bled 200 cc.											
5/8	440	668	459	203	30.3	66	0.85	3,9	12,0	10.90	61	
5/8	Fasting											
5/14	620	802	515	283	35.3	77	0.73	5,3	12,2	10.30	78	
5/14	Diet: 100 grams dried, ground white bread, 500 cc. skim milk											
5/21	638	852	560	270	31.7	75	0.73	5,1	17,0	9.95	96	* Poik. +
5/28	633	891	582	299	33.6	71	0.66	5,4	14,2	9.90	90	* Poik. +
6/4	798	929	574	345	37.2	86	0.63	6,8	10,6	9.75	95	* Poik. +

* Poikilocytosis of red cells.

No previous anemia experiments on this dog.

The experiments given in tables 30 and 31 are very similar and may be discussed together. In both experiments the amount of bread and milk was not measured, but it is significant that there was a slight but definite loss of body weight during the bread and milk periods. It is to be expected, therefore that the diet would not suffice to raise the level of hemoglobin and pigment volume much above the anemia

level. The larger dog (table 30) does show a slow regeneration toward normal in 4 weeks, but the smaller dog (table 31) shows almost no net gain during 3 weeks. There is a little gain in the first week which is lost subsequently.

The mixed diet reaction is very nicely shown in both experiments (tables 30 and 31) and a week or two is sufficient to make up the deficit in hemoglobin and establish the normal level.

TABLE 30

Blood regeneration—bread and milk. Dog 18-113. Bull mongrel, female, age 4 to 5 months

DATE, 1918	PIGMENT VOLUME = Hb. PER CENT TIMES BLOOD VOLUME	BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	R. B. C. HEMATOCRIT	Hb.	COLOR INDEX	R. B. C. MILLION	W. B. C.	WEIGHT	BLOOD PER KILOGRAM	REMARKS
		cc.	*cc.*	*cc.*	*per cent*	*per cent*				*kgm.*	*cc.*	
4/24	970	1000	561	426	42.6	97	0.63	7,7	9,6	9.75	103	
4/24	Diet: Bread and milk—amount not measured											
4/25	Bled 250 cc.											
4/26	Bled 170 cc No distress											
4/29	418	853	559	277	32.5	49	0.52	4.7	12,6	9.50	90	
5/8	597	776	468	295	38.0	77	0.61	6.3	8,6	9.13	85	
5/15	878	1140	674	445	39.1	77	0.49	7,9	9,6	9.10	125	*Fragm.
5/22	666	912	555	341	37.3	73	0.52	7,0	10,2	9.30	98	*Fragm.
5/29	701	788	473	315	40.0	89	0.55	8,1	9,8	9.10	87	*Fragm.++
5/29	Diet: Mixed diet											
6/10	940	940	530	397	42.2	100	0.60	8,4	10,2	10.45	90	*Fragm.

* Fragmentation of red cells.

No previous anemia experiments on this dog.

A *bile fistula* experiment is included (table 32) to show that these dogs react like normal dogs as regards blood pigment production after simple anemia. This dog was known to have complete exclusion of bile from the intestinal tract (autopsy notes). His general condition during the entire experiment was excellent and the bread and milk diet was sufficient to maintain the body weight close to normal. During the entire period there was a loss of only 1.4 kilos, which is not

great when we consider the size of the dog and the length of the experiment (7 weeks). There was a slight initial gain in hemoglobin and pigment volume which was subsequently lost. The general level of pigment volume, red cell hematocrit and hemoglobin is pretty nearly uniform with occasional temporary gains and losses. It is of interest to note a steady gain in number of red cells from 3,400,000 to 6,000,000. The hemoglobin gain was much less, which gives a fall in color index

TABLE 31

Blood regeneration—bread and milk. Dog 18-115. Bull mongrel, female, young adult

DATE, 1918	PIGMENT VOLUME = Hb. PERCENT TIMES BLOOD VOLUME	BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	R. B. C. HEMATOCRIT	Hb.	COLOR INDEX	R. B. C. MILLION	W. B. C.	WEIGHT	BLOOD PLATELETS/CMM	REMARKS
		cc.	*cc.*	*cc.*	*per cent*	*per cent*				*kgm.*	*c.*	
4/24	655	780	394	368	47.2	84	0.59	7.1	14,2	7.00	111	
4/24	Diet: Bread and milk—amount not measured											
4/25	Bled 195 cc.											
4/26	Bled 140 cc.											
4/29	314	582	375	200	34.3	54	0.63	4.3	26.2	7.50	80	
5/8	572	752	467	274	36.45	76	0.59	6.5	9.4	6.77	111	
5/15	675	888	522	353	39.8	76	0.59	6.4	25.0	6.40	138	
5/22	468	593	354	233	39.3	79	0.70	5.6	6.0	6.20	96	
5/23	Diet: Mixed diet											
5/29	516	607	373	228	37.5	85	0.64	6.6	10.8	6.90	88	[a] Poik.
6/10	769	761	438	318	41.8	101	0.73	6.9	14.2	8.00	95	[a] Poik.

[a] Poikilocytosis of red cells.

No previous anemia experiments on this dog.

from 0.87 to 0.54. This is not uncommon in normal dogs under similar experimental conditions.

Crackermeal, milk, lard and butter. These crackermeal experiments were performed in part during the war period when white bread was not available. This crackermeal consisted of an unknown mixture including at least wheat, barley and rice flours, possibly others. The experiments show reactions which resemble accurately those observed

with white bread. A mixture of grain flours, therefore, is no more efficient in promoting a regeneration of hemoglobin and red cells than white bread alone, consisting mainly of wheat flour.

Table 33 is to be compared with table 30—the first experiment done with white bread and milk, and the second with crackermeal and milk.

TABLE 32

Blood regeneration—bread and milk diet—bile fistula. Dog 17–151. White bull mongrel, male, young adult

DATE, 1917	PIGMENT VOLUME = Hb. PER CENT TIMES BLOOD VOLUME	BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	R. B. C. HEMATOCRIT	Hb.	COLOR INDEX	R. B. C. MILLION	W. B. C.	WEIGHT	BLOOD PER KILOGRAM
		cc.	*cc.*	*cc.*	*per cent*	*per cent*				*kgm.*	*cc.*
5/28	2675	2158	971	1085	55	124	0.72	8,6	5,2	18.20	113
5/28	Diet: Bread and milk										
5/29	Bled 540 cc.										
5/30	Bled 540 cc.										
5/31	830	1408	1000	408	29	59	0.87	3,4	14,6	17.50	80
5/31	Diet: Bread and milk—amount not measured										
6/8	1058	1557	1059	498	32	68	0.77	4,4	9,8	17.20	90
6/15	1575	1852	1222	630	34	85	0.65	6,5	6,6	16.80	110
6/22	1274	1464	937	527	36	87	0.63	6,9	10,8	16.40	89
6/29	990	1415	920	495	35	70	0.49	7,2	7,4	16.10	88
7/6	1150	1532	950	582	38	75	0.54	6,9	5,8	15.90	96
7/13	1480	1741	1097	644	37	85	0.55	7,7	13,8	15.90	109
7/18	1118	1574	1039	545	34	71	0.54	6,6	13,8	16.10	98

Blood volume with dry oxalate. Hemoglobin by Sahli tubes.
No previous anemia experiments on this dog.

These experiments were performed during a period of rapid growth and the diets in each instance were sufficient to preserve body weight. There was a slight gain in weight during the crackermeal experiment. Both experiments show a slow steady gain in pigment volume, hemoglobin and red cell hematocrit. We may say the curves are as nearly identical as one can hope to observe in this type of experiment.

After a period of 8 weeks of crackermeal and milk diet the dog suddenly developed acute *dietary deficiency disease* which resulted in

TABLE 33

Blood regeneration—crackermeal and milk. Dog 18–113. Bull mongrel, female, age 8 to 9 months

DATE, 1918	PIGMENT VOLUME = Hb. PER CENT TIMES BLOOD VOLUME	BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	R. B. C. HEMATOCRIT	Hb.	COLOR INDEX	R. B. C. MILLION	W. B. C.	WEIGHT	BLOOD PER KILOGRAM	REMARKS
		cc.	*cc.*	*cc.*	*per cent*	*per cent*				*kgm.*	*cc.*	
8/14	1556	1136	571	557	49.0	137	0.81	8,4	5,2	12.85	88	
8/15	Diet: Bread and milk											
8/16	Bled 284 cc.											
8/17	Bled 284 cc.											
8/19	714	978	699	270	27.6	73				12.45	79	
8/19	Bled 220 cc.											
8/21	804	1200	858	323	26.9	67	0.84	4,0	26,2	12.35	97	
8/21	Diet: 200 grams crackermeal, 500 cc. milk											
8/27	788	984	667	308	31.3	80	0.80	5,0	12,4	12.50	79	* Poik.
9/4	894	1048	670	367	35.0	85	0.66	6,4	8,6	12.55	84	* Poik. ++
9/11	1078	1135	688	441	38.9	95	0.65	7,3	6,4	12.55	90	* Poik. ++
9/19	1136	1152	700	442	38.4	99	0.67	7,4	18,0	12.60	92	* Poik. ++
9/27	1380	1315	737	558	42.5	105	0.67	7,8	14,8	12.90	102	* Poik. +
10/9	1085	1119	640	497	44.4	97	0.63	7,7	11,0	12.80	93	
10/17	1290	1277	728	536	42.0	101				12.75	100	
10/18	Diet: Mixed diet. Extra meat. Dietary deficiency disease											
10/25	788	1050	690	355	33.8	75	0.58	6,5	10,8	10.35	101	
10/26	Killed. Autopsy											

* Poikilocytosis of red cells.

Refer to table 30, bread and milk experiment.

death 1 week later. Note the fall in blood volume, hemoglobin and red cell hematocrit during this short period. The autopsy findings will not be discussed at this time.

Table 34 presents a long experiment in which the diet is crackermeal, lard and butter in sufficient amounts to preserve the body weight and permit of a gain of 0.7 kilo during the period of 12 weeks. No previous anemia experiments had been performed on this dog, which

TABLE 34

Blood regeneration—crackermeal, lard and butter. Dog 17–205. Bull mongrel, male, young adult

DATE, 1917	PIGMENT VOLUME = Hb. PER CENT TIMES BLOOD VOLUME	BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	R. B. C. HEMATOCRIT	Hb.	COLOR INDEX	R. B. C. MILLION	W. B. C.	WEIGHT	BLOOD PER KILOGRAM
		cc.	*cc.*	*cc.*	*per cent*	*per cent*				*kgm.*	*cc.*
10/29	1450	1058	571	487	46	137	0.87	7,9	8,6	10.70	99
10/29	Diet: Bread and milk										
10/30	Bled 265 cc.										
10/31	Bled 265 cc.										
11/2	522	790	563	229	29	66	1.14	2,9	27,0	10.40	76
11/2	Diet: 200 grams crackermeal, 10 grams lard, 10 grams butter										
11/9	559	860	585	275	32	65			6,2	10.30	83
11/16	948	1088	664	425	39	87	1.01	4,3	18,8	10.30	105
11/23	969	1052	589	463	44	92	0.90	5,1	9,2	10.30	102
11/28	998	998	549	449	45	100	0.78	6,4	9,2	10.30	97
12/5	1111	1028	555	473	46	108	0.86	6,3	8,8	10.40	99
12/10	1070	1008	544	464	46	106	0.82	6,5	13,6	10.20	99
12/19	904	913	511	404	44	99	0.77	6,4	13,6	10.60	86
12/26					44	119	0.79	7,5	8,0	10.60	
1/2/18					48	124	0.78	7,9	10,2	10.50	
1/9					46	127	0.79	8,0	12,8	10.70	
1/17					46	127	0.85	7,5	14,2	10.70	
1/23	1480	1139	592	546	48	130	0.69	9,4	13,4	11.10	103

No previous anemia experiments on this dog.
Blood volume with dry oxalate. Hemoglobin by Sahli tubes.

may account for the fact that the blood regeneration finally carried the level to normal. This reserve has been mentioned in the preceding communication. The gain is very slow and at times there appears to be a slight loss in red cell hematocrit or hemoglobin or pigment volume.

The end result after 12 weeks of this diet may be accepted as normal. It is unusual that this dog tolerated this diet for such a prolonged period without any signs of dietary deficiency disease. A subsequent experiment (paper V) shows dietary deficiency symptoms in this same dog after a shorter period of a similar diet.

TABLE 35

Blood regeneration—crackermeal, lard and butter. Dog 16-160. White bull mongrel, female, age 12 months

DATE, 1917	PIGMENT VOLUME = Hb. PER CENT TIMES BLOOD VOLUME	BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	R. B. C. HEMATOCRIT	Hb.	COLOR INDEX	R. B. C. MILLION	W. B. C.	WEIGHT	BLOOD PER KILOGRAM	REMARKS
		cc.	*cc.*	*cc.*	*per cent*	*per cent*				*kgm.*	*cc.*	
3/4	1218	1006	533	473	47	121	0.67	9,1	7,2	10.00	100	
3/4	Diet: Crackermeal, lard and butter											
3/6	Bled 262 cc.											
3/7	Bled 242 cc.											
3/9	726	844	591	256	30	86	0.86	5,0	9,4	9.60	89	
3/9	Diet: 206 grams crackermeal, 10 grams lard, 10 grams butter											
3/15	732	842	581	261	31	87	0.72	6,0	8,4	9.30	91	
3/20	762	786	517	267	34	97	1.08	4,5	9,8	9.30	84	
3/27	840	785	526	259	33	107	0.89	6,0	8,6	9.20	85	Slight diarrhea
4/3	812	805	531	274	34	101	0.83	6,1	15,8	8.90	90	
4/9	845	836	535	301	36	101	0.78	6,5	12,6	9.10	92	

Experimental history, see table 18-b.
Blood volume with dry oxalate. Hemoglobin by Sahli tubes.

The experiment given in table 35 is very much like the preceding one but for the fact that this dog had been observed previously in anemia periods (see experimental history 18-b). The blood regeneration is very slow on the same amount of crackermeal, lard and butter. There was a slight loss of weight on this diet which contains a sufficient number of calories per kilo. This is to be explained by the presence of diarrhea.

The addition of milk powder (table 36) to the lard, butter and crackermeal diet does not modify the curve of blood regeneration nor does it prevent the development of the dietary deficiency disease after a period of 1 month. The peculiar reaction of the red cell hematocrit which actually diminishes as the hemoglobin and red cell count increase may be explained in part by the use of dry oxalate in varying amounts.

TABLE 36

Blood regeneration—crackermeal, lard, butter and milk powder. Dog 16–140. Bull mongrel, male, young adult

DATE, 1918	PIGMENT VOLUME = Hb. PER CENT TIMES BLOOD VOLUME	BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	R. B. C. HEMATOCRIT	Hb.	COLOR INDEX	R. B. C. MILLION	W. B. C.	WEIGHT	BLOOD PER KILOGRAM	REMARKS
		cc.	*cc.*	*cc.*	*per cent*	*per cent*				*kgm.*	*cc.*	
3/4	2212	1616	711	905	56	137	0.77	8,9	6,8	15.10	107	
3/4	Diet: Crackermeal, lard and butter											
3/6	Bled 414 cc.											
3/7	Bled 394 cc.											
3/9	1027	1222	880	465	38	84	0.87	4,8	13,2	14.30	85	
3/9	Diet: 163 grams crackermeal, 10 grams lard, 10 grams butter, 100 ± grams milk powder											
3/15	1215	1322	846	476	36	92	0.88	5,2	11,2	14.8	89	
3/20	1153	1281	883	448	35	90	0.88	5,1	9,6	15.0	85	
3/27	1055	1227	834	392	32	86	0.84	5,1	8,0	14.80	83	
4/3	1350	1324	834	490	37	102	0.77	6,6	19,8	14.60	91	
4/9	1408	1257	855	402	32	112	0.84	6,7	11,6	14.10	89	*

* Dietary deficiency disease. Death April 17.

Blood volume with dry oxalate. Hemoglobin by Sahli tubes.

The pigment volume regeneration is about the expected amount in view of the diet which was sufficient to maintain but not increase the body weight.

The next three experiments may be discussed in a group (tables 37, 38 and 39). The influence of splenectomy is concerned in two of these experiments and under these experimental conditions the blood regeneration appears to progress in a normal fashion, at least for a time.

TABLE 36-B

Experimental history. Dog 16-140

EXPERIMENT NUMBER	DIET	BLOOD REGENERATION		WEIGHT	REMARKS
		Pigment volume	Blood per kilogram		
				kgm.	
Begin 9/13/16	Mixed			8.1	
Bled 450 cc.				8.4	(3 bleedings)
End 10/13/16				10.1	
Begin 11/6/16	Fasting, metabolism			9.3	
Bled 660 cc.	Sugar, metabolism			8.2	(4 bleedings)
End 12/16/16	Gliadin, sugar Gelatin, sugar			6.4	
Begin 9/11/17	Sugar, metabolism	1570	109	13.1	
Bled 712 cc.	Mono-amino-acid	756	90	12.6	
End 10/19/17	fraction of gelatin	1273	130	9.3	
Begin 3/4/18	Crackermeal, lard,	2210	107	15.1	Table 36
Bled 808 cc.	butter, milk pow-	1025	85	14.3	
End 4/9/18	der	1385	89	14.1	Dietary deficiency disease

TABLE 37

Blood regeneration—crackermeal, lard, butter, alfalfa meal. Dog 18-97. Bull mongrel, female, young adult

DATE, 1918	PIGMENT VOLUME = Hb. PER CENT TIMES BLOOD VOLUME	BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	R. B. C. HEMATOCRIT	Hb.	COLOR INDEX	R. B. C. MILLION	W. B. C.	WEIGHT	BLOOD PER KILOGRAM
		cc.	*cc.*	*cc.*	*per cent*	*per cent*				*kgm.*	*cc.*
3/11	2038	1358	584	774	57	150	0.88	8,5	9,2	12.40	109
3/11	Diet: Bread and milk										
3/13	Bled 339 cc.										
3/14	Bled 340 cc.										
3/16	908	966	570	396	41	94	1.04	4,5	15,6	11.40	82
3/16	Diet: Crackermeal, lard, butter, 40 grams alfalfa meal										
3/22	1175	1118	548	570	51	105	0.86	6,1	22,2	10.70	103
3/29	1250	1058	561	498	47	118	0.75	7,9	14,6	10.80	98
4/4	1322	1076	581	495	46	123	0.74	8,3	12,4	10.90	99
4/10	1598	1102	595	508	46	145	0.75	9,7	11,4	11.00	100

Blood volume with dry oxalate. Hemoglobin by Sahli tubes.

No previous anemia experiments on this dog.

TABLE 38

Blood regeneration—crackermeal, lard and butter—splenectomy. Dog 17-163. Bull mongrel, male, young adult

DATE, 1917	PIGMENT VOLUME = Hb. PER CENT TIMES BLOOD VOLUME	BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	R. B. C. HEMATOCRIT	Hb.	COLOR INDEX	R. B. C. MILLION	W. B. C.	WEIGHT	BLOOD PER KILOGRAM	REMARKS
		cc.	*cc.*	*cc.*	*per cent*	*per cent*				*kgm.*	*cc.*	
9/3	1110	1069	588	481	45	104	0.72	7.2	6,2	10.0	106	
9/3	Diet: Bread and milk											
9/4	Bled 279 cc.											
9/5	Bled 279 cc.											
9/7	388	719	532	187	26	54	0.90	3,0	22,4	9.70	74	
9/7	Diet: 200 grams crackermeal, 20 grams lard											
9/14	610	813	512	301	37	75	1.01	3,7	10,2	9.60	85	
9/21	728	867	512	355	41	84		5,8	5,6	9.60	91	
9/22	Diet: 200 grams crackermeal, 10 grams lard, 10 grams butter											
9/28	754	820	492	328	40	92	0.72	6,7	8,0	9.60	85	
10/5	704	800	456	344	43	88	0.73	6,0	13,4	9.80	80	
10/12	806	848	517	331	39	95	0.82	5,8	5,6	9.70	87	
10/17	886	914	539	375	41	97	0.88	5,5	6,6	9.70	94	
10/25	868	914	585	329	36	95	0.79	6,0	8,0	9.80	93	
10/31	145	596	518	78	13	24	0.75	1,6	20,4	9.70	61	*

* Death. (Internal hemorrhages, urobilin in urine ++.)

Blood volume by dry oxalate. Hemoglobin by Sahli tubes.

TABLE 38-B

Experimental history. Dog 17-163 (splenectomy)

EXPERIMENT NUMBER	DIET	BLOOD REGENERATION		WEIGHT	REMARKS
		Pigment volume	Blood per kilogram		
				kgm.	
Begin 4/24/17	Meat, Blaud's pills	918	102	9.0	Table 75
Bled 458 cc.		340	76	8.9	
End 6/6/17		1063	117	8.6	
Begin 9/3/17	Crackermeal, lard	1112	106	10.0	Table 38
Bled 558 cc.	Crackermeal, lard,	388	74	9.7	
9/21/17	butter	728	91	9.6	
10/25/17		868	93	9.8	
End 10/31/17		143	61	9.7	Death 11/1/17

Table 38 illustrates a not infrequent condition which develops in splenectomized dogs made anemic and fed on a limited diet. We have pointed out elsewhere (1) that there is a remarkable condition which may develop in splenectomized bile fistula dogs. In these bile fistula dogs if anemia is produced we may observe periods of spontaneous blood destruction and enormous pigment overproduction. Under such conditions it was suggested that the body was forming its maxi-

TABLE 39

Blood regeneration—crackermeal, lard, butter, alfalfa meal—splenectomy. Dog 17–34. Bull mongrel, female, young adult

DATE, 1918	PIGMENT VOLUME = Hb. PER CENT TIMES BLOOD VOLUME	BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	R. B. C. HEMATOCRIT	Hb.	COLOR INDEX	R. B. C. MILLION	W. B. C.	WEIGHT	BLOOD PER KILOGRAM
		cc.	*cc.*	*cc.*	*per cent*	*per cent*				*kgm.*	*cc.*
3/11	1776	1345	740	606	45	132	0.94	7,0	11,8	16.10	84
3/11	Diet: Bread and milk										
3/13	Bled 336 cc.										
3/14	Bled 336 cc.										
3/16	828	1034	765	269	26	80	1.14	3,5	22,0	15.30	68
3/16	Diet: Crackermeal, lard, butter, 50 grams alfalfa meal										
3/22	1128	1128	722	407	36	100	1.00	5,0	17,4	14.80	76
3/29	1282	1187	760	427	36	108	0.93	5,8	17,0	15.20	78
4/4	1185	1162	732	430	37	102	0.77	6,6	16,2	15.00	77
4/10	1371	1193	740	453	38	115	1.08	5,3	16,8	14.80	81

Blood volume with dry oxalate. Hemoglobin by Sahli tubes.
Experimental history, see table 17–b.

mum amounts of pigment material (hemoglobin as well as bile pigment) but the red cell stroma was lacking in quantity or quality. It was suggested that the spleen might be concerned in the development of red cell stroma. These present exeriments may point to the production of faulty red cell stroma under such conditions (table 38) but some readers may wish to postulate the development of some unknown poison to explain the distintegration of the red cells in our splenectomy experiments. This condition develops very abruptly and may super-

vene in a dog with relatively normal red cell count and hemoglobin values. Within 2 or 3 days the red cell count may fall to one-third normal (table 38) and abnormal pigments appear in blood serum and urine. Much more study must be given to this condition and we expect to report further work in this line.

Alfalfa meal was added to the diets in two of these experiments. These experiments give no evidence to indicate that alfalfa meal exerts a definite influence upon the curve of hemoglobin regeneration in the dog. The alfalfa meal used in our experiments was the usual grade of finely ground alfalfa purchased on the open market.

Rice, potatoes and milk. We may consider the next group of experiments as a unit (tables 40, 41, 42, 43 and 44). In principle all these experiments are similar and the results are remarkably uniform. In the first four experiments the dogs were bled and placed upon a uniform diet of cooked rice, boiled potatoes and skim milk. The regeneration in most of the experiments was slow but uniform with the end result after 5 to 6 weeks about normal or slightly below the normal blood level. After this there followed a short period (7 to 10 days) of mixed diet. Then a second period of anemia and blood regeneration upon the same rice, potato, milk diet was observed. These second periods are replicas of the first regeneration periods on this same diet.

It is clear that a liberal diet of cooked rice and potato with skim milk sufficient to maintain or slightly increase the body weight will give a slow steady gain in blood pigment, red cell hematocrit, red cell count, etc., which will often bring the regeneration curve back to normal or close to normal.

Two experiments are exceptions to the general reaction (tables 40 and 44). Table 40 shows a regeneration which is incomplete and not back to normal in 5 weeks. In fact, during the last month the regeneration is not in evidence and the pigment volume, hemoglobin and red cell hematocrit are stationary. There was a slight loss of weight during this period but the dog was very active and normal in all respects. The second anemia regeneration period shows an identical reaction. Table 44 shows a still more striking difference from the normal average regeneration. This dog refused to eat the amounts of rice and potato and milk given at first. She ate the amounts recorded in table 44, which amount to about 50 per cent that given to the other dogs, or 50 calories per kilo body weight. During 6 weeks there was a loss of 2.5 kilos and the blood regeneration was only slight during this whole period.

TABLE 40

Blood regeneration—rice, potatoes and milk—repeat experiment. Dog 19-104. Bull mongrel pup, male

DATE, 1919	PIGMENT VOLUME = Hb. PER CENT TIMES BLOOD VOLUME	BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	R. B. C. HEMATOCRIT	Hb.	COLOR INDEX	R. B. C. MILLION	W. B. C.	WEIGHT	BLOOD PER KILOGRAM	REMARKS
		cc.	*cc.*	*cc.*	*per cent*	*per cent*				*kgm.*	*cc.*	
1/30	1367	1067	455	602	56.4	128				8.75	122	
2/6	1394	1078	468	594	55.1	129	0.68	9,5	14,8	9.00	120	
2/6	Diet: Bread and milk											
2/7	Bled 270 cc.											
2/8	Bled 270 cc. No distress											
2/10	363	630	457	170	26.9	58	0.76	3,8	25,8	8.35	75	
2/10	Diet: 363 grams rice, 417 grams potatoes, 500 cc. milk											
2/17	692	765	451	311	40.6	90	0.82	5,5	11,6	8.00	96	
2/26	933	859	451	404	47.0	109	0.75	7,3	8,4	8.00	107	
3/3	807	816	445	366	44.9	99	0.70	7,1	10,2	7.90	103	* Poik.+†
3/10	910	833	419	410	49.2	109	0.80	6,8	10,0	7.85	106	* Poik.
3/19	814	773	402	362	46.9	105	0.67	7,8	9,0	7.85	98	* Poik.+‡
3/21	Diet: Mixed diet. Extra food											
3/31	1036	898	433	456	50.8	115	0.69	8,3	12,2	8.65	104	
3/31	Diet: Bread and milk											
4/1	Bled 225 cc.											
4/2	Bled 225 cc. No distress											
4/3	462	688	462	223	32.4	67	0.88	3,8	24,8	8.20	84	
4/3	Diet: 363 grams rice, 417 grams potatoes, 500 cc. milk											
4/11	656	780	469	300	38.4	84	0.70	6,0	11,2	8.20	97	* Poik.
4/18	701	772	435	330	42.7	91	0.56	8,1	8,8	8.05	96	
4/25	760	764	421	339	44.4	98	0.55	8,9	8,0	8.00	95	
5/2	844	816	421	391	47.9	103	0.64	8,1	7,8	7.90	103	
5/9	791	804	431	369	45.9	98	0.58	8,5	5,8	7.65	105	

* Poikilocytosis of red cells.

† Only 300 cc. of milk given.

‡ Gave 300 grams rice and 300 grams potatoes.

No previous anemia experiments on this dog.

TABLE 41

Blood regeneration—rice, potatoes and milk—repeat experiment. Dog 19–95. Bull mongrel, male, age 5 months

DATE, 1919	PIGMENT VOLUME = Hb. PERCENTAGE BLOOD VOLUME	BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	R. B. C. HEMATOCRIT	Hb.	COLOR INDEX	R. B. C. MILLION	W. B. C.	WEIGHT	BLOOD PER KILOGRAM	REMARKS
		cc.	*cc.*	*cc.*	*per cent*	*per cent*				*kgm.*	*cc.*	
1/16	1232	1130	595	528	46.4	109	0.67	8,2	18,0	11.35	100	
1/16	Diet: Crackermeal and milk											
1/17	Bled 282 cc.											
1/18	Bled 282 cc. No distress											
1/20	560	824	602	218	26.4	68	0.77	4,4	30,4	11.00	75	
1/20	Diet: 418 grams boiled rice, 490 grams potatoes, 500 cc. milk											
1/27	742	974	574	395	40.6	76	0.72	5,3	16,2	12.75	76	
2/3	954	976	531	435	44.6	98	0.65	7,5	22,8	11.15	87	
2/12	982	1013	538	464	45.8	97	0.75	6,5	14,2	10.90	93	
2/19	1040	1000	511	480	48.0	104	0.70	7,4	10.6	10.60	95	
2/28	1242	1058	514	538	50.9	118	0.88	6.7	9,8	10.80	98	* Poik.+
3/7	983	1006	521	475	47.2	98	0.87	7.2	10.0	11.20	90	* Poik.+
3/10	Diet: Mixed diet											
3/17	1092	1130	640	484	42.8	97	0.84	5,8	13,0	12.80	88	* Poik.
3/17	Diet: Crackermeal and milk											
3/18	Bled 283 cc.											
3/19	Bled 283 cc. No distress											
3/21	570	934	664	251	26.9	61	0.92	3,3	18,0	12.45	75	* Poik.
3/21	Diet: 418 grams boiled rice, 490 grams potatoes, 500 cc. milk											
3/28	577	834	576	250	30.0	69	0.86	4.0	6,0	11.75	79	* Poik.
4/2	741	953	605	334	35.0	78	0.63	6,2	11,6	11.80	81	* Poik.+
4/9	773	976	600	371	38.0	79	0.70	5,6	8,2	11.85	82	
4/16	988	1046	588	448	42.8	94	0.69	6,8	10,4	11.90	98	
4/23	1085	1073	576	492	45.8	101	0.68	7,4	20.0	11.95	90	
4/30	1237	1168	576	581	49.7	106	0.65	8,1	8,2	11.95	98	

* Poikilocytosis of red cells.

No previous anemia experiments on this dog.

TABLE 42

Blood regeneration—rice, potatoes and milk—repeat experiment. Dog 19-33. Bull mongrel, female, age 5 months

DATE, 1919	PIGMENT VOLUME = Hb. PER CENT TIMES BLOOD VOLUME	BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	R. B. C. HEMATOCRIT	Hb.	COLOR INDEX	R. B. C. MILLION	W. B. C.	WEIGHT	BLOOD PER KILOGRAM	REMARKS
		cc.	*cc.*	*cc.*	*per cent*	*per cent*				*kgm.*	*cc.*	
1/16	1435	1380	742	616	44.7	104	0.59	8,9	18,6	10.95	126	
1/16	Diet: Bread and milk											
1/17	Bled 345 cc.											
1/18	Bled 245 cc. No distress											
1/20	594	886	640	237	26.8	67	0.76	4,4	15,2	10.25	86	
1/20	Diet: 400 grams boiled rice, 475 grams potatoes, 500 cc. milk											
1/27	768	1084	638	436	40.2	71	0.48	7,4	10,8	10.35	105	* Slight
2/3	1058	1080	570	494	45.7	98	0.60	8,1	15,2	9.55	113	* Slight Cells small
2/12	1040	1063	566	482	45.3	98	0.69	7,1	8.4	9.90	107	*
2/19	1245	1163	570	582	50.0	107	0.73	7,3	6,4	9.55	122	* Slight
2/28	1255	1125	568	540	48.0	112	0.84	6.7	5,8	9.65	116	* Slight
3/7	1098	1127	568	542	48.1	98	0.73	6,7	6,0	10.35	113	* Slight
3/10	Diet: Mixed diet											
3/17	1040	1004	516	478	47.6	104	0.70	7.4	7,8	11.35	89	* Slight
3/17	Diet: Bread and milk											
3/18	Bled 251 cc.											
3/19	Bled 251 cc. No distress											
3/21	566	916	647	260	28.4	62	0.84	3,7	12,2	11.15	82	*
3/21	Diet: 400 grams boiled rice, 475 grams potatoes, 500 cc. milk											
3/28	896	1028	614	397	38.6	87	0.85	5,1	7,8	11.35	90	
4/2	908	1085	631	433	39.9	84	0.65	6,5	8,6	11.20	97	* Slight
4/9	858	990	568	412	41.6	87	0.60	7,2	6,0	11.35	87	
4/16	1136	1112	565	530	47.7	102	0.67	7,6	8,8	11.30	98	* Slight
4/23	1212	1142	568	557	48.8	106	0.57	9,3	6,8	11.35	100	
4/30	1313	1198	572	604	50.4	110	0.58	9,5	7,4	11.15	107	* Slight

* Poikilocytosis of red cells.

No previous anemia experiments on this dog.

TABLE 43

Blood regeneration—rice, potatoes and milk—repeat experiment. Dog 19–103. Bull mongrel, female, age 5 months

DATE, 1919	PIGMENT VOLUME = Hb. PER CENT TIMES BLOOD VOLUME	BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	R. B. C. HEMATOCRIT	Hb.	COLOR INDEX	R. B. C. MILLION	W. B. C.	WEIGHT	BLOOD PER KILOGRAM	REMARKS
		cc.	*cc.*	*cc.*	*per cent*	*per cent*				*kgm.*	*cc.*	
1/30	1100	1077	588	483	44.9	102				9.55	113	
2/6	978	1020	598	428	41.9	96	0.61	7,9	12,6	10.00	102	
2/6	Diet: Bread and milk											
2/7	Bled 255 cc.											
2/8	Bled 255 cc. No distress											
2/10	430	808	600	204	25.3	53	0.78	3,4	9,0	9.45	85	
2/10	Diet: 411 grams rice, 472 grams potatoes, 500 cc. milk											
2/17	770	854	485	365	42.7	90	0.78	5,8	12,4	8.85	96	
2/26	996	981	520	446	45.5	102	0.68	7,5	10,8	9.10	108	
3/3	1305	1070	491	574	53.6	122	0.80	7,6	8,4	9.20	116	
3/10	1068	1008	526	476	47.3	106	0.68	7,8	7,0	9.20	110	
3/19	1065	986	499	477	48.4	108	0.72	7,5	9,0	9.60	103	*
3/21	Diet: Mixed diet. Extra food											
3/31	1103	1103	600	492	44.6	100	0.71	7,0	10,6	10.65	103	
3/31	Diet: Bread and milk											
4/1	Bled 276 cc.											
4/2	Bled 276 cc. No distress											
4/3	444	837	612	220	26.3	53	0.76	3,5	11,6	10.40	80	
4/3	Diet: 411 grams rice, 472 grams potatoes, 500 cc. milk											
4/11	756	964	603	347	36.0	79	0.76	5,2	8,8	10.70	90	
4/18	803	957	570	377	39.4	84	0.57	7,4	14,6	10.55	91	
4/25	970	1003	546	439	43.8	97	0.64	7,6	7,2	10.65	94	
5/2	1077	1057	560	491	46.5	102	0.62	8,2	11,0	10.35	102	
5/9	1045	1062	592	460	43.3	98	0.65	7,5	14,6	10.50	101	

* Poikilocytosis of red cells.

No previous anemia experiments on this dog.

This experiment (table 44) shows admirably a reaction noted in other experiments. The bleeding reduced the hemoglobin from 123 per cent to 59 per cent and the red cells from 7,500,000 to 3,300,000. During the 6 weeks' observation we note a rise of only 24 per cent hemoglobin but the red count returns to normal. The color index of course

TABLE 44

Blood regeneration—rice, potatoes and milk. Dog 19-96. Bull mongrel, female, age 8 months

DATE, 1919	PIGMENT VOLUME = Hb. PER CENT TIMES BLOOD VOLUME	BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	R. B. C. HEMATOCRIT	Hb.	COLOR INDEX	R. B. C. MILLION	W. B. C.	WEIGHT	BLOOD PER KILOGRAM	REMARKS
		cc.	*cc.*	*cc.*	*per cent*	*per cent*				*kgm.*	*cc.*	
3/17	1520	1238	568	663	53.6	123	0.82	7,5	11,4	13.50	92	
3/17	Diet: Crackermeal and milk											
3/18	Bled 310 cc.											
3/19	Bled 310 cc. No distress											
3/21	527	891	636	246	27.6	59	0.89	3,3	14,2	12.40	72	
3/21	Diet:† 200 grams boiled rice, 200 grams potatoes, 500 cc. milk											
3/28	616	835	551	267	32.0	74	0.93	4,0	14,8	11.55	79	
4/2	662	911	598	308	33.4	73	0.65	5,6	11,4	11.20	81	* Poik. ++
4/9	784	959	581	368	38.4	82	0.59	6,9	10,4	10.75	89	* Poik. +
4/16	728	908	550	354	39.0	80	0.56	7,1	6,8	10.30	88	* Poik. +
4/23	818	905	532	368	40.7	90	0.61	7,4	10,2	10.10	90	* Poik. ++
4/30	778	938	535	394	42.0	83	0.58	7,2	12,0	9.90	95	* Poik. ++

* Poikilocytosis of red cells.

† Animal refused to eat larger quantities of food. Represents about 50 calories per kilo of body weight.

Experimental history, see table 4-b.

drops from 0.93 to 0.58 and poikilocytosis is very much in evidence. Under such conditions one feels a very strong probability of red cell fragmentation.

This evidence (tables 40 and 44) confirms our belief that the *amount of any diet* may be a considerable factor in blood regeneration. Given a diet of a limited nature but sufficient to permit of slight gain in body

weight and we may expect a certain amount of blood regeneration, at times even a return to normal. But given a limited diet in small amounts not sufficient for maintenance of body weight, we may confidently expect a very slow blood regeneration or complete absence of active regeneration. Under such circumstances the body may even be unable to make up its blood cell maintenance factor and the pigment volume curve may actually fall. This is a favorable time for

TABLE 45

Blood regeneration—casein, sugar, butter and lard. Dog 18-56. Bull mongrel, female, young adult

DATE, 1917	PIGMENT VOLUME = Hb. PER CENT TIMES BLOOD VOLUME	BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	R. B. C. HEMATOCRIT	Hb.	COLOR INDEX	R. B. C. MILLION	W. B. C.	WEIGHT	BLOOD PER KILOGRAM
		cc.	*cc.*	*cc.*	*per cent*	*per cent*				*kgm.*	*cc.*
10/29	1275	930	400	530	57	137	0.89	7,7	12,6	8.40	111
10/29	Diet: Bread and milk										
10/30	Bled 233 cc.										
10/31	Bled 233 cc.										
11/2	340	548	400	148	27	62	1.20	2,6	16,0	7.50	73
11/2	Diet: 75 grams casein, 25 grams sugar, 20 grams butter, 20 grams lard										
11/9	428	586	375	211	36	73	1.30	2,8	19,6	7.50	73
11/16	786	827	463	364	44	95	0.99	4,8	6,0	8.10	102
11/23	725	763	412	351	46	95	0.93	5,1	12,0	7.20	106
11/28	908	810	389	421	52	112	0.81	6,9	12,2	7.30	111
12/5	1330	985	384	601	61	135	0.85	7,9	13,2	7.40	133

Blood volume with dry oxalate. Hemoglobin by Sahli tubes.
No previous anemia experiments on this dog.

the appearance of a characteristic dietary deficiency disease which is much like scurvy in human beings and is rapidly fatal if not energetically treated with antiscorbutic measures.

Casein and gliadin. When we consider a bread and milk diet from the standpoint of dietary factors we are obviously dealing with many known and unknown constituents. Two of the familiar ingredients in this bread and milk diet are casein and gliadin, which are concerned

particularly in the following group of experiments (tables 45 to 47 inclusive). Casein used in these experiments was obtained from a large dairy products company in this state. It appears as a fine dry granular powder, pale yellow in color, and is of reasonable purity, judging from information given us by the chemist of this company.

The gliadin was extracted from wheat flour in this laboratory by use of dilute alcohol (70 per cent). The weighed amount of gliadin was thoroughly mixed with the sugar, moistened with water and fed to the dog by spoon. Total ingestion was readily accomplished in this way.

Table 45 shows the influence of casein, sugar, lard and butter on blood regeneration. The diet was sufficient to maintain body weight and the blood regeneration was complete in 5 weeks. We must not forget that this dog had not been used for anemia experiments previous to this time and such dogs occasionally show remarkable regenerative capacity on limited diets. The next experiment, however, is conclusive and shows the effect of casein under more carefully controlled and less favorable conditions.

The second casein experiment (table 46) is preceded by a 2 weeks' sugar diet period during which the expected reaction is noted. There is the usual gain in red cell hematocrit, hemoglobin and pigment volume. If the sugar diet had been continued we are reasonably certain that the pigment volume would have remained stationary or even have fallen. Casein added to the diet shows a distinct gain which is held during the subsequent weeks when we see slight fluctuations in pigment volume but relatively little change. There is a slight gain in weight as the calories in the diet are increased by the use of fats. The figures given for the urinary nitrogen show the normal level for the sugar periods and during the sugar and casein intervals indicate the amount of nitrogenous metabolism.

Table 47 is to be compared with a preceding experiment (table 21, paper II). In both experiments a gliadin sugar diet is used over a period of several weeks. This experiment shows less conclusive evidence of the influence of gliadin upon blood regeneration. We may conclude that the gliadin in this experiment (table 47) was without influence on the curve of blood regeneration. We are inclined to the opinion, however, that sugar alone over this period in this experiment would have been associated with a definite loss in pigment volume by the end of the 6-week period. This experiment shows a transient increase in pigment volume which is lost during the last 2 weeks.

TABLE 46

Blood regeneration—casein, sugar, lard and butter. Dog 16–158. Coach mongrel, male, young adult

DATE, 1917	PIGMENT VOLUME = Hb. PER CENT TIMES BLOOD VOLUME	BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	R. B. C. HEMATOCRIT	Hb.	COLOR INDEX	R. B. C. MILLION	W. B. C.	WEIGHT	BLOOD PER KILOGRAM	AVERAGE DAILY URINARY NITROGEN	REMARKS
		cc.	*cc.*	*cc.*	*per cent*	*per cent*				*kgm.*	*cc.*	*grams*	
10/17	1817	1367	588	779	57	133	0.74	8.9	11,0	9.70	141		
10/17	Diet: Bread and milk												
10/19	Bled 342 cc.												
10/21	Bled 342 cc.												
10/22	576	823	576	247	30	70	0.95	3,7	11,2	9.50	87		
10/22	Diet: 75 grams cane sugar, 25 grams glucose, 300 cc. water												
10/29	707	895	573	312	36	79	0.79	5,0	10,6	8.60	104	1.81	
11/5	766	958	575	383	40	80	0.67	6,0	6,0	7.90	121	1.61	
11/5	Diet: 100 grams sugar, 50 grams casein, 300 cc. water												
11/12	856	961	567	394	41	89	0.73	6,1	3,8	7.80	123	4.70	
11/19	1038	1081	616	465	43	96	0.67	7,2	5,2	7.70	140	6.60	Vomiting
11/21	Diet: 100 grams sugar, 100 grams casein, 300 cc. water												
11/26	878	944	538	406	43	93	0.69	6,7	5.0	7.80	121	9.36	
12/3	824	970	582	388	40	85	0.75	5,7	6.2	8.00	121	11.60	
12/3	Diet: 100 grams sugar, 100 grams casein, 10 grams lard, 10 grams butter												
12/10	876	974	604	370	38	90	0.57	7,9	9,8	8.30	117		
12/19	876	952	600	352	37	92	0.69	6,7	14,0	8.70	108		
12/20	Diet: 100 grams sugar, 125 grams casein, 10 grams lard, 10 grams butter												
12/26					40	96	0.61	7,8	9,0	8.90			
1/2/18					38	93	0.55	8,4	7,8	9.10			
1/9					35	92	0.62	7,4	8,6	9.30			
1/17					38	85	0.50	8,5	6,8	9.10			
1/23	975	975	614	361	37	100	0.58	8,7	6,2	8.90	109		

Blood volume with dry oxalate. Hemoglobin by Sahli tubes.

TABLE 46-B

Experimental history. Dog 16-158

EXPERIMENT NUMBER	DIET	BLOOD REGENERATION		WEIGHT	REMARKS
		Pigment volume	Blood per kilogram		
				kgm.	
Begin 9/26/16	Meat			8.1	
Bled 600 cc				7.7	(4 bleedings)
End 11/20/16				9.5	Complete regeneration of Hb. and R.B.C.
Begin 2/12/17	Beef heart	1523	147	9.9	Table 56
Bled 726 cc.		385	85	9.8	
End 4/11/17		1476	152	8.8	
Begin 5/7/17	Sugar, gliadin	1505	146	9.0	Table 47
Bled 660 cc.	Metabolism	458	84	8.4	
End 6/18/17		456	113	6.2	R.B.C. fragmented, shadow forms
Begin 10/17/17	Sugar	1818	141	9.7	Table 46
Bled 684 cc.	Sugar and casein	576	87	9.5	
End 12/3/17	Metabolism	825	121	8.0	
Begin 5/3/18	Sugar, glycocoll	1800	119	11.7	
Bled 698 cc.	Metabolism	528	76	10.9	
End 6/18/18		852	99	8.05	Maximum regeneration 3 weeks, then drop
Begin 8/28/18	Sugar and gelatin	974	82	10.3	
Bled 929 cc.		548	88	9.15	(5 bleedings)
End 9/30/18		508	90	7.45	Maximum regeneration 2 weeks
Begin 2/20/19	Sugar and liver residue	1862	111	12.35	Table 67
Bled 934 cc.		478	70	11.10	(3 bleedings)
End 3/18/19		738	82	9.15	
Begin 8/18/19	Beet tops	1468	93	12.55	
Bled 851 cc.	Spinach	560	77	12.10	(3 bleedings)
End 10/24/19		785	86	10.50	

These experiments are not conclusive but give evidence that moderate amounts of gliadin with sugar alone do not modify profoundly the blood regeneration curve. That gliadin in combination with other factors may have a more favorable influence on red cell production may be granted. Also under the conditions of the experiment it appears that casein has a more favorable influence on blood regeneration than gliadin when given with sugar only.

TABLE 47

Blood regeneration—sugar and gliadin. Dog 16–158. Coach mongrel, male, young adult

DATE, 1917	PIGMENT VOLUME = Hb. PER CENT TIMES BLOOD VOLUME	BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	R. B. C. HEMATOCRIT	Hb.	COLOR INDEX	R. B. C. MILLION	W. B. C.	WEIGHT	BLOOD PER KILOGRAM	REMARKS
		cc.	*cc.*	*cc.*	*per cent*	*per cent*				*kgm.*	*cc.*	
5/7	1505	1320	581	740	56	114	0.57	10.1	6,6	9.00	146	
5/8	Bled 330 cc.											
5/9	Bled 330 cc.											
5/11	458	705	480	226	32	65	0.64	5,1	10,8	8.40	84	
5/11	Diet: 50 grams cane sugar, 25 grams dextrose, 25 grams gliadin, 300 cc. water											
5/14	434	804	547	257	32	54	0.68	4,0	9,0	8.20	98	
5/21	435	791	538	253	32	55	0.59	4,7	8,6	7.80	101	*
5/28	596	961	519	346	36	62	0.56	5,5	9,8	7.40	129	
6/4	604	863	552	311	36	70	0.61	5,7	8,4	6.90	122	
6/11	559	766	528	237	31	73	0.61	6,0	9,2	6.60	111	*
6/18	456	702	477	225	32	65	0.53	6,1	11,6	6.20	113	*

* Fragmentation of red blood cells.

Blood volume with dry oxalate. Hemoglobin by Sahli tubes.

Experimental history, see table 46–b.

SUMMARY

A diet of dried white bread and skim milk may cause a slow, steady gain in blood pigment volume from week to week. A liberal diet of this type sufficient to maintain or increase body weight will often suffice for complete blood regeneration. A restricted diet of bread and

milk barely sufficient for body maintenance will rarely permit of complete blood regeneration following simple secondary anemia.

Repeat experiments done after short intervals of rest to permit complete return to normal condition will show identical reactions on the part of the hemoglobin, red cells and pigment volume. The animal shows no increased ability to produce hemoglobin and red cells after repeated experiments nor is there any evidence for a failure of red cell production under these conditions.

Bile fistula dogs presenting complete exclusion of bile pigments from the intestine show a reaction which is practically identical with that of normal dogs.

Crackermeal (a mixture of wheat flour, barley flour and rice flour) with milk or lard and butter, gives a blood pigment reaction following anemia which is similar to the familiar bread and milk reaction.

A dietary deficiency disease may develop in these dogs kept on limited diets for many weeks. This condition clinically resembles scurvy in human beings and may be prevented or cured by antiscorbutic measures. This question will be reviewed in a subsequent publication.

Splenectomy may not modify the expected reaction of red blood cells following anemia. In certain splenectomy experiments there develops a peculiar condition associated with spontaneous destruction or disintegration of circulating red cells. This may appear following a limited diet of several weeks and runs a very rapid course resulting in death within a few days.

Rice, potatoes and skim milk make up a diet which may be classed with bread and milk as regards its influence upon red blood cell regeneration following the unit hemorrhages. If anything, this diet is slightly more efficient than bread and milk in promoting blood regeneration.

Casein and gliadin by themselves are not efficient factors in promoting red cell regeneration but casein appears to be the more efficient in the amounts used and under the conditions of these experiments.

Any one of these diet mixtures in proper amounts may be used to maintain the pigment volume at a constant level following the initial 2 weeks' blood reaction. Under such conditions any added food factor may be measured with some accuracy as to its power of aiding in blood regeneration..

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BLOOD REGENERATION FOLLOWING SIMPLE ANEMIA

IV. Influence of Meat, Liver and Various Extractives, Alone or Combined with Standard Diets *

G. H. WHIPPLE, F. S. ROBSCHEIT AND C. W. HOOPER

From the George Williams Hooper Foundation for Medical Research, University of California Medical School, San Francisco

Received for publication April 3, 1920

Cooked meat and liver stand in striking contrast to the milk, bread, potato mixtures outlined in the preceding paper of this series. Cooked liver, lean beef or beef heart alone or in combination are very efficient in bringing about a rapid blood regeneration following the standard type of secondary anemia. These substances are very similar but for the present we may say their efficiency is in the order given; that is, cooked liver is most effective in anemia provided a sufficient amount (caloric value) is eaten, and cooked beef heart is least effective; but the differences are not great and this order may be changed with the accumulation of more data.

These three substances are efficient in stimulating blood regeneration whether given alone or in combination, or together with carbohydrate or mixed diets. They all stand the severe test of promoting definite blood regeneration when administered after long limited diet periods unfavorable to blood regeneration.

Meat extract (commercial) has no value in the blood regeneration complex. But a watery liver extract seems to exert a distinct influence on the blood regeneration. Liver residue (after the watery and alcoholic extraction) alone exerts a definite influence on blood regeneration. We do not wish to go into a discussion of this question of tissue extracts until we present much more experimental data dealing with this and other material of similar nature.

EXPERIMENTAL OBSERVATIONS

In general the experimental technique has been detailed in the first paper of this series. All meat and liver were cooked thoroughly in boiling water before feeding, with the exception of "meat scraps."

The meat scraps were obtained from the University Hospital and included meats of various kinds cooked in different ways. A certain amount of fat was of necessity included in this meat diet. Unless otherwise noted these diets were completely ingested. With an occasional exception noted in the tables the dogs were in uniformly excellent condition.

TABLE 48

Blood regeneration—cooked meat scraps. Dog 17–38. Bull mongrel, female, adult

DATE, 1920	PIGMENT VOLUME = Hb. PER CENT TIMES BLOOD VOLUME	BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	R. B. C. HEMATOCRIT	Hb.	COLOR INDEX	R. B. C. MILLION	W. B. C.	WEIGHT	BLOOD PER KILOGRAM
		cc.	*cc.*	*cc.*	*per cent*	*per cent*				*kgm.*	*cc.*
2/12	1322	1268	670	591	46.6	104	0.71	7.3	7.4	10.90	115
2/12	Diet: Bread and milk										
2/13	Bled 317 cc.										
2/14	Bled 317 cc.										
2/16	550	1000	714	281	28.1	55	0.75	3.7	6.2	10.35	97
2/16	Diet: 500 grams cooked meat scraps										
2/24	948	1168	713	435	37.2	80	0.69	5.8	10.8	11.05	106
3/1	1046	1236	742	485	39.2	85	0.66	6.4	5.8	11.50	107
3/8	1437	1332	682	636	47.8	108	0.68	7.9	9.8	12.10	110
3/15	1332	1378	734	626	45.4	97	0.62	7.8	7.4	12.85	107
3/22	1475	1420	704	704	49.5	104	0.62	8.4	8.2	13.20	108

Experimental history, see table 20-b.

The first two tables show the characteristic reaction to a diet of meat scraps. There is a prompt and rapid regeneration of hemoglobin and red cells which brings the hematocrit and pigment volume back to practically normal in 3 weeks. This level is sustained for the subsequent 2 weeks. The first experiment (table 48) shows a rather low initial level (104 per cent hemoglobin) but a very prompt reaction following the anemia. The hemoglobin, red cell hematocrit and pigment volume return to a level slightly above the original normal level. There was a marked gain in weight.

The second experiment (table 49) required a third bleeding to reduce the red cells to the usual anemia level. The regeneration of the red cells is practically complete in 3 weeks, although the previous high level of hemoglobin and red cell hematocrit is not reached. This level is uniform during the next 2 weeks. In both experiments the plasma volume is relatively constant during the entire period of observation.

TABLE 49

Blood regeneration—cooked meat scraps. Dog 18–114. Bull mongrel, female, adult

DATE, 1920	PIGMENT VOLUME = Hb. PER CENT TIMES BLOOD VOLUME	BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	R. B. C. HEMATOCRIT	Hb.	COLOR INDEX	R. B. C. MILLION	W. B. C.	WEIGHT	BLOOD PER KILOGRAM
		cc.	*cc.*	*cc.*	*per cent*	*per cent*				*kgm.*	*cc.*
2/12	1995	1705	766	931	54.6	117	0.70	8,4	10,8	15.50	110
2/12	Diet: Bread and milk										
2/13	Bled 425 cc.										
2/14	Bled 425 cc.										
2/16	822	1232	818	403	32.7	67	0.98	3,6	7,8	14.30	86
2/16	Bled 275 cc.										
2/18	Diet: 600 grams cooked meat scraps										
2/24	1135	1480	950	504	34.0	77	0.80	4,8	10,2	14.25	104
3/1	1434	1525	857	640	42.0	94	0.73	6,4	9,0	14.35	106
3/8	1580	1540	798	726	47.2	103	0.68	7,6	8,2	15.15	102
3/15	1678	1678	874	796	47.4	100	0.63	7,9	7,8	15.50	108
3/22	1787	1728	838	864	50.0	103	0.63	8,2	9,4	15.50	111

Experimental history, see table 12–b.

Table 50 presents a reaction very much like that noted in the two preceding experiments. Fresh lean beef in adequate amounts was fed. It was cooked in boiling water. This meat contained very little fat and was purchased under the trade name of "chuck." The dog shows a very high level before bleeding and three bleedings did not reduce the level to the usual anemia level. This dog had not been used previously in anemia experiments. The blood regeneration was rapid in the first

2 weeks, but there was little gain in the third week except in the red count, which shows a remarkable jump. This gives a corresponding fall in the color index. A change to a mixed diet shows but little change until the third week of mixed diet when the original high level was attained.

TABLE 50

Blood regeneration—lean beef. Dog 18–24. Bull mongrel, male, young adult

DATE, 1918	PIGMENT VOLUME = Hb. PER CENT TIMES BLOOD VOLUME	BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	R. B. C. HEMATOCRIT	Hb.	COLOR INDEX	R. B. C. MILLION	W. B. C.	WEIGHT	BLOOD PER KILOGRAM
		cc.	*cc.*	*cc.*	*per cent*	*per cent*				*kgm.*	*cc.*
11/14	1560	1300	480	814	62.6	120	0.87	8.4	12.0	13.20	99
11/12	Diet: Crackermeal and milk										
11/15	Bled 325 cc. Slight distress										
11/16	Bled 195 cc.										
11/18	582	915	612	298	32.6	64				12.70	72
11/18	Bled 150 cc.										
11/20	466	806	539	264	32.7	58	0.81	3.6	20.2	11.85	68
11/20	Diet: 567 grams cooked lean beef										
11/27	935	995	590	399	40.1	94	0.80	5.9	15.8	12.70	78
12/4	1093	1062	585	473	44.5	103	0.61	8.4	14.9	13.15	81
12/11	1090	1033	582	466	45.1	106	0.48	11.9	15.0	13.40	77
12/13	Diet: Mixed diet										
12/20	1055	1115	594	511	45.8	95	0.47	10.1	25.8	13.50	83
12/27	1010	1060	544	516	48.6	95				13.20	80
1/10/19	1425	1230	550	674	54.8	116	0.58	10.1	7.8	13.20	93

No previous anemia experiments on this dog.

Table 51 illustrates a reaction which is in no sense typical, but complicated by abnormal factors. It is submitted with reservations. This dog has been used in a variety of blood regeneration experiments (table 6-b), therefore the type of reaction is well established. But within a few weeks following the present experiment the dog died with bilateral

TABLE 51

Blood regeneration—lean beef and gelatin—lean beef and brain. Dog 17–28. Bull mongrel, female, young adult

DATE, 1918 1919	PIGMENT VOLUME = Hb. PER CENT TIMES BLOOD VOLUME	BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	R. B. C. HEMATOCRIT	Hb.	COLOR INDEX	R. B. C. MILLION	W. B. C.	WEIGHT	BLOOD PER KILOGRAM	REMARKS
		cc.	*cc.*	*cc.*	*per cent*	*per cent*				*kgm.*	*cc.*	
12/2	2118	1650	740	903	54.7	128	0.58	11.0	9.8	15.70	105	
12/2	Diet: Crackermeal and milk											
12/3	Bled 413 cc.											
12/4	Bled 413 cc.											
12/6	1000	1256	850	394	31.3	79				15.30	82	
12/7	Bled 314 cc.											
12/9	708	1240	926	300	24.2	57	0.79	3.6	15.6	15.05	82	
12/9	Diet: 681 grams cooked lean beef, 20 grams cooked gelatin—100 calories per kilo											
12/16	916	1063	636	388	36.5	86	0.84	5.1	26.2	13.35	80	See footnote
12/17	Diet: 100 grams cooked brain, 580 grams lean beef—100 calories per kilo											
12/23	954	1047	607	412	39.4	91	0.53	8.5	19.0	12.90	81	See footnote
12/23	Diet: Mixed diet											
12/30	883	1100	722	366	33.3	80				13.35	82	
1/8/19	1162	1263	720	531	42.0	92	0.69	6.7	10.2	13.50	94	* Poik. ++
1/15	1050	1290	750	530	41.0	82	0.64	6.4	10.6	14.05	92	*
1/22	1380	1450	792	635	43.8	95	0.64	7.4	17.4	14.65	99	

* Poikilocytosis of red blood cells.

December 16, 1918: Food not touched. Seems sick. Drank considerable water this a.m. and immediately afterward vomited it. Temperature, 38.4°C. Abdomen seems distended. Gave 150 grams meat and 50 grams crackermeal.

December 23, 1918: Left 300 grams food. Very thirsty, vomits water within 5 minutes after drinking. Temperature, 38.8°C. Inactive, slight dragging of right hind leg. Put in metabolism cage; 400 cc. water; mixed diet.

December 26, 1918: Animal recovered. Is still thirsty.

Subsequent death. Stone in kidney.

Experimental history, see table 6–b; see autopsy, table 14.

renal calculi. It is therefore certain that this dog was suffering from renal disease during this experiment (table 51). We note in this experiment that the dog lost weight rapidly on the diet of beef and gelatin which was not eaten with relish. Finally the diet was changed as

TABLE 52

Blood regeneration—beef heart and liver. Dog 18–116. Bull mongrel, female, young adult

DATE, 1918-1919	PIGMENT VOLUME = Hb PER CENT TIMES BLOOD VOLUME	BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	R. B. C. HEMATOCRIT	Hb	COLOR INDEX	R. B. C. MILLION	W. B. C.	WEIGHT	BLOOD PER KILOGRAM
		cc.	*cc.*	*cc.*	*per cent*	*per cent*				*kgm.*	*cc.*
12/2/18	2120	1720	805	907	52.7	123	0.56	11,4	14.1	14.90	115
12/2	Diet: Crackermeal and milk										
12/3	Bled 430 cc.										
12/4	Bled 430 cc.										
12/6	939	1183	803	369	31.2	79	0.57	6,9	21,2	14.55	81
12/7	Bled 300 cc.										
12/9	776	1260	930	323	25.6	62	0.84	3,7	17,5	13.95	90
12/9	Diet: 256 grams cooked beef heart,* 610 grams cooked beef liver*—100 calories per kilo										
12/16	1082	1330	844	476	35.8	81	0.88	4,6	18,2	14.55	91
12/23	1890	1685	860	818	48.5	112	0.57	9,9	11,5	15.25	110
12/30	2270	1648	747	902	54.7	138				15.50	106
12/30	Diet: Mixed diet										
1/8/19	2040	1715	785	912	53.2	119	0.72	8,3	7,0	15.50	110
1/15	1760	1610	762	842	52.2	109	0.68	8,0	12,0	16.20	99
1/22	2120	1740	773	948	54.4	122	0.71	8,6	10,6	15.65	111

* Meat cooked, fat and connective tissue removed, and ground.
Experimental history, see table 13-b.

the beef and gelatin mixture was refused. The beef and brain mixture was also eaten poorly, and more weight was lost. We are fortunate in being able to refer to a fasting experiment on this same dog (table 14) which shows practically an identical gain during two weeks as recorded

in table 51. The distaste for the food mixture which was eaten only in part we believe is largely responsible for this lack of blood regeneration. We see that the renal disease does not modify the expected blood reaction during a fasting period (table 14) and we have no right to assume that it might seriously modify the reaction in table 51.

TABLE 53

Blood regeneration—beef heart. Dog 19–6. Bull mongrel, male, young adult

DATE, 1918–1919	PIGMENT VOLUME = Hb. PER CENT TIMES BLOOD VOLUME	BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	R. B. C. HEMATOCRIT	Hb.	COLOR INDEX	R. B. C. MILLION	W. B. C.	WEIGHT	BLOOD PER KILOGRAM
		cc.	*cc.*	*cc.*	*per cent*	*per cent*				*kgm.*	*cc.*
11/14	2082	1543	590	946	61.3	135	0.77	8,8	11,0	14.00	110
11/14	Diet: Crackermeal and milk										
11/15	Bled 386 cc.										
11/16	Bled 386 cc. No distress										
11/18	800	1020	650	365	35.8	78				13.00	79
11/18	Bled 200 cc.										
11/20	697	1026	710	317	30.8	68	0.74	4,6	16,4	12.80	80
11/20	Diet: 431 grams cooked beef heart*—100 calories per kilo										
11/27	1325	1250	670	574	45.9	106	0.79	6,7	6,0	12.55	100
12/4	1480	1170	568	601	51.1	126	0.64	9,8	6,7	12.75	92
12/11	1642	1325	637	681	51.4	124	0.62	10,5	10,2	12.25	108
12/13	Diet: Mixed diet										
12/20	1376	1188	588	588	49.5	116	0.58	10,0	11,4	12.50	95
12/27	1287	1226	642	579	47.2	105				13.15	93
1/10/19	1940	1462	607	840	57.5	133	0.81	8,2	7,0	13.20	110

* Beef heart cooked, fat and connective tissue removed, and ground.
No previous anemia experiments on this dog.

Table 52 illustrates an optimum reaction on a diet of beef heart and beef liver. Both these substances favor a rapid blood regeneration especially when a sufficient amount is eaten. This dog ate the mixture with relish and gained over 1 kilo during 3 weeks. There is a truly

remarkable gain in red cells, hemoglobin and pigment volume. During *3 weeks* the regeneration is complete and the anemia level (3 bleedings) if anything was below the average level. Many other anemia experiments have been completed on this dog (table 13-b) and the type

TABLE 54

Blood regeneration—beef heart. Dog 19–84. Bull mongrel pup, female

DATE, 1918-1919	PIGMENT VOLUME = Hb. PER CENT TIMES BLOOD VOLUME	BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	R. B. C. HEMATOCRIT	Hb.	COLOR INDEX	R. B. C. MILLION	W. B. C.	WEIGHT	BLOOD PER KILOGRAM	REMARKS
		cc.	*cc.*	*cc.*	*per cent*	*per cent*				*kgm.*	*cc.*	
12/2	992	780	352	424	54.4	127	0.53	12.0	10.6	6.95	112	R. B. C. small
12/2	Diet: Crackermeal and milk											
12/3	Bled 195 cc. No distress											
12/4	Bled 125 cc. No distress											
12/6	448	491	305	183	37.3	91	0.68	6.7	24.0	6.10	80	
12/7	Bled 123 cc.											
12/9	428	542	360	177	32.6	79	1.20	3.3	17.1	5.85	93	
12/9	Diet: 228 grams beef heart*—100 calories per kilo											
12/16	638	608	332	274	45.0	105	0.86	6.1	10.1	5.80	105	
12/23	796	676	328	345	51.0	118	0.59	10.8	15.2	5.90	115	R. B. C. small
12/30	968	711	323	387	54.5	136				5.90	120	
12/30	Diet: Mixed diet											
1/8/19	855	750	366	380	50.5	114	0.71	8.0	23.8	6.95	107	
1/15	712	818	405	409	50.0	87	0.61	7.1	10.8	7.80	105	
1/22	1020	937	476	456	48.7	109	0.55	10.5	14.2	7.50	125	
1/30	987	888	429	455	51.2	111	0.75	7.4	11.2	7.50	118	

* Beef heart cooked, fat and connective tissue removed, ground.

No previous anemia experiments on this dog.

reaction is therefore established. It may be stated even that the regeneration was *almost complete within 2 weeks.*

With the change to mixed diet we note a reaction which is not uncommon when a sudden change is made from a fixed diet to another

very different diet. Under such conditions even when the second diet is most favorable we may record a slight fall in red cell hematocrit, hemoglobin and pigment volume. This experiment, too, shows a fall in the red count. We have no good explanation to offer, but this fact is to be considered in the proper interpretation of various tables.

TABLE 55

Blood regeneration—beef heart and lean meat following 3 weeks of sugar—metabolism. Dog 16–157. Bull mongrel, male, age 10 months

DATE, 1916-1917	PIGMENT VOLUME = Hb PER CENT TIMES BLOOD VOLUME	BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	R. B. C. HEMATOCRIT	Hb.	COLOR INDEX	R. B. C. MILLION	W. B. C.	WEIGHT	BLOOD PER KILOGRAM	REMARKS
		cc.	*cc.*	*cc.*	*per cent*	*per cent*				*kgm.*	*cc.*	
12/14/16						116		7.9	6,4	8.10		
12/14	Diet: Sugar (3 weeks)											
1/5/17	546	976	625	351	36	56	0.55	5,1	7,2	6.10	160	
1/5	Diet: 400 grams beef heart, 100 grams sugar											
1/11					43	76	0.58	6.6	11,8	6.60		
1/13	Diet: Lean meat											
1/17	408	908	563	345	38	45	0.44	5,1	6,4	7.60	119	*
1/26	738	838	478	360	43	88	0.59	7,5	8,2	8.00	105	*
2/2	838	998	529	469	47	84	0.57	7,3	7,0	8.20	122	
2/16	1130	1202	553	649	54	94	0.53	8,8	9,8	9.20	131	
2/23	1052	1052	526	526	50	100	0.58	8,6	13,4	9.20	115	
3/2	1208	1220	549	672	55	99	0.56	8,9	9,8	9.20	132	

* Anisocytosis and poikilocytosis of red blood cells.

Blood volume with dry oxalate. Hemoglobin by Sahli tubes.

Cooked beef heart is a food commonly used in experimental laboratories. It is usually assumed that heart muscle as a food is very like skeletal muscle, although there may be certain differences as pointed out by Mendel and Osborne (1). We have found that it compares favorably with skeletal muscle as far as concerns the regeneration of red cells and hemoglobin. Whether beef heart is actually identical with lean beef in its effect on blood regeneration cannot be stated posi-

tively as we cannot at this time submit a sufficiently complete series of controlled experiments.

Tables 53 and 54 are identical in all essential factors. Both dogs were used for the first time and we must consider the unusual" reserve" which at times may be demonstrated by such dogs. The cooked beef heart was eagerly eaten, and the weight was practically stationary, although the first dog lost a little during the third week. Blood regeneration was practically complete in 3 weeks as regards red cells,

TABLE 55-B

Experimental history. Dog 16-157

EXPERIMENT	DIET	BLOOD REGENERATION		WEIGHT	REMARKS
		Pigment volume	Blood per kilogram		
				kgm.	
Begin 9/26/16	Fasting			7.90	
Bled 600 cc.				6.80	(4 bleedings)
End 10/11/16				5.40	Slight regeneration of Hb. and R. B. C.
Begin 12/14/16	Sugar, metabolism			8.10	
Bled 600 cc.		1208	132	7.30	(4 bleedings)
End 3/2/17	Sugar, beef heart Beef heart			9.20	
Begin 5/7/17	Gelatin, sugar	1570	130	10.00	
Bled 648 cc.	Metabolism	429	74	9.20	
End 6/11/17		792	126	6.30	
6/14	Killed—bled from carotid				

hemoglobin and pigment volume. Both dogs showed a distinct drop when changed to a mixed diet,—a change recorded in red cell hematocrit, hemoglobin and red count. There was no change in plasma volume to explain this fluctuation and for the present we must be content with recording this fact without advancing any convincing explanation (refer to exper. 52). A similar fall is noted in table 66 even when the change is from a poor diet (bread and milk) to a more favorable diet (beef heart).

Table 55 illustrates the reaction on beef heart diet following a 3-week period of sugar feeding. After the meat diet is established we note a slow but steady gain back to normal in 4 to 5 weeks. We wish to emphasize the fact that a period of fasting or sugar feeding makes subsequent blood regeneration much more difficult and this is a severe test for any food substance. Only meat (including beef heart) and

TABLE 56

Blood regeneration—beef heart. Dog 16–158. Coach mongrel, male, young adult

DATE, 1917	PIGMENT VOLUME = Hb. PER CENT TIMES BLOOD VOLUME	BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	R. B. C. HEMATOCRIT	Hb.	COLOR INDEX	R. B. C. MILLION	W. B. C.	WEIGHT	BLOOD PER KILOGRAM
		cc.	*cc.*	*cc.*	*per cent*	*per cent*				*kgm.*	*cc.*
2/12	1525	1451	566	886	61	105	0.63	8.3	7.0	9.90	147
2/12	Diet: Bread and milk										
2/13	Bled 363 cc.										
2/14	Bled 363 cc.										
2/15	385	837	560	276	33	46	0.70	3.3	7.4	9.80	85
2/15	Diet: Beef heart										
2/21	500	980	647	333	34	51	0.77	3.3	10.8	9.70	101
2/28	602	885	523	363	41	68	0.68	5.0	19.8	9.80	90
3/7	840	1077	560	517	48	78	0.56	6.9	19.8	9.80	109
3/14	904	1062	531	531	50	85	0.57	7.5	14.0	9.30	114
3/21	1235	1272	585	687	54	93	0.60	7.8	10.0	9.50	134
3/28	1146	1180	543	638	54	97	0.58	8.3	8.0	9.50	124
4/5	1280	1243	572	671	54	103	0.56	9.2	8.6	9.30	134
4/11	1478	1342	604	738	55	110	0.58	9.4	18.6	8.80	152

Experimental history, see table 46–b.

liver show up to advantage as compared with the mixed diet under such conditions. Apparently the fasting or sugar feeding or other limited diet causes a draining of the body's reserve and subsequent blood regeneration suffers because of this depletion of reserve or impairment of function.

Tables 56 and 57 are incomplete in that the amount of beef heart is not known but the weights give assurance that a liberal amount of food

was consumed. There is only a trifling loss of weight during the last week of the experiment when the blood picture had returned to normal. It is obvious that the blood regeneration in these two experiments is much slower than that recorded in experiments 53 and 54. Four to 6 weeks elapse before the hemoglobin and hematocrit return to normal and 5 to 7 before the pigment volume and red cell count return to the original level.

TABLE 57

Blood regeneration—beef heart. Dog 17–192. Bull mongrel, male, young adult

DATE, 1917	PIGMENT VOLUME = Hb. PER CENT TIMES BLOOD VOLUME	BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	R. B. C. HEMATOCRIT	Hb.	COLOR INDEX	R. B. C. MILLION	W. B. C.	WEIGHT	BLOOD PER KILOGRAM
		cc.	*cc.*	*cc.*	*per cent*	*per cent*				*kgm.*	*cc.*
4/24	1157	1032	537	496	48	112	0.78	7.2	10.6	9.80	105
4/24	Diet: Bread and milk										
4/25	Bled 258 cc.										
4/26	Bled 258 cc.										
4/27	444	822	633	189	23	54	0.84	3.2	12.8	9.50	87
4/27	Diet: Beef heart										
5/2	662	883	609	274	31	75	0.79	4.7	11.6	9.90	89
5/9	804	992	585	407	41	81	0.67	6.0	8.0	9.50	104
5/16	1030	1050	567	483	46	98	0.80	6.1	12.0	9.50	110
5/23	1255	1172	633	539	46	107	0.73	7.3	9.0	9.50	123
5/30	1308	1147	585	562	49	114	0.81	7.0	8.0	9.50	121
6/6	1360	1214	607	607	50	112	0.64	8.7	6.8	9.10	133

No previous anemia experiments on this dog.
Blood volume with dry oxalate. Hemoglobin by Sahli tubes.

Tables 58 and 59 both deal with bile fistula dogs. The experiments were performed at the same time under identical conditions and the reaction to the beef heart diet is strikingly uniform. It is known from the autopsy notes that the bile was completely excluded from the intestine. A meat diet is not well tolerated by these bile fistula dogs over long periods and a loss of weight is usually noted under such conditions. As a result of this long period of meat feeding we note subsequent intoxication which resulted fatally in spite of a mixed diet régime.

The beef heart was eaten with relish but the amounts given are not recorded. There is a distinct gain in hemoglobin, red cells and pigment volume during each of the first 3 weeks. The level at the end of

TABLE 58

Blood regeneration—beef heart—bile fistula. Dog 17–35. Bull mongrel, female, young adult

DATE, 1917	PIGMENT VOLUME = Hb. PER CENT TIMES BLOOD VOLUME	BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	R. B. C. HEMATOCRIT	Hb.	COLOR INDEX	R. B. C. MILLION	W. B. C.	WEIGHT	BLOOD PER KILOGRAM	REMARKS
		cc.	*cc.*	*cc.*	*per cent*	*per cent*				*kgm.*	*cc.*	
5/28	1330	1244	560	684	55	107	0.74	7,2	20,0	9.10	136	
5/29	Bled 311 cc.											
5/30	Bled 311 cc.											
5/31						48	0.77	3,1	45,6	9.10		
5/31	Diet: Beef heart											
6/8	540	900	567	333	37	60	0.79	3,8	14,6	8.20	109	
6/15	868	923	480	443	48	94	0.78	6,0	7,0	8.40	109	
6/22	952	933	476	457	49	102	0.72	7,1	8,4	8.10	115	
6/29	800	1000	590	410	41	80	0.74	5,4	6,8	8.40	119	No increase in bile pigment
7/6	649	729	459	277	38	89	0.78	5,7	7,0	8.00	91	
7/9	Diet: Mixed diet											
7/13	694	846	533	313	37	82	0.77	5,3	20,0	8.80	96	
7/18	797	848	500	348	41	94	0.90	5,2	19,8	9.00	94	
8/9	Death from bile fistula intoxication											

Bile pigment daily output per 6 hours before this experiment (28 day average) = 14.0 mgm.

Bile pigment daily output per 6 hours during this experiment (30 day average) = 13.4 mgm.

Blood volume with dry oxalate. Hemoglobin by Sahli tubes.

the third week is almost normal in both dogs and if the experiments had been terminated at this point we should of necessity conclude that the reaction was similar to that so often observed in the normal dog.

But the fourth week in both dogs shows a decided drop in pigment volume, hemoglobin and red cell hematocrit.

We fortunately have the daily *bile pigment output figures* at hand and can say that there is no increase in bile pigment elimination during this week of falling hemoglobin. So we may not explain this decrease in the curve as due to some agent destructive to the red cells. It has been established (2) that a sudden destruction of red cells in the blood stream will result in an increased output of bile pigment although the reaction is not in any degree a quantitative reaction as some observers have claimed (3). We have no convincing explanation for these observed facts but suggest that the poor quality of the red cell may be a

TABLE 58–8

Experimental history. Dog 17–35 (bile fistula)

EXPERIMENT	DIET	BLOOD REGENERATION		WEIGHT	REMARKS
		Pigment volume	Blood per kilogram		
				kgm.	
Begin 12/18/16	Lean meat			8.80	100 Hb.
Bled 600 cc.				8.90	(4 bleedings)
End 1/17/17		892	99	8.90	101 Hb.
3/8/17	Bile fistula operation				
Begin 5/28/17	Beef heart	1330	136	9.10	107 Hb.
Bled 622 cc.				9.10	
End 7/6/17		649	91	8.00	Maximum regeneration 3 weeks

factor. Limited diets as well as splenectomy under certain experimental conditions seem to be associated with red cells which are prone to disintegrate more readily than normal.

The average daily bile pigment output is given in each table for a 30-day period before the experiment and during the anemia regeneration period. One dog shows identical figures for the mixed diet control and beef heart period. The other dog shows a much higher output on the mixed diet than on the beef heart diet. We believe this is to be explained by the mixed diet which is made up of meat scraps, bones, bread, potatoes, table scraps, etc., and is given in moderate excess. This allows a certain choice on the part of the dog and if the animal prefers the carbohydrate fractions we may observe the familiar reac-

tion to carbohydrate feeding in the bile fistula dog, namely, an increase in bile pigments.

TABLE 59

Blood regeneration—beef heart—bile fistula. Dog 17–155. Bull mongrel, male, young adult

DATE, 1917	PIGMENT VOLUME = Hb. PER CENT TIMES BLOOD VOLUME	BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	R. B. C. HEMATOCRIT	Hb.	COLOR INDEX	R. B. C. MILLION	W. B. C.	WEIGHT	BLOOD PER KILOGRAM	REMARKS
		cc.	*cc.*	*cc.*	*per cent*	*per cent*				*kgm.*	*cc.*	
5/28	1560	1416	708	708	50	110	0.67	8,2	11,2	11.50	123	
5/28	Diet: Mixed diet											
5/29	Bled 354 cc.											
5/30	Bled 354 cc.											
5/31	495	935	645	290	31	53	0.63	4,2	9,2	10.80	86	
5/31	Diet: Boiled beef heart											
6/8	1030	1213	752	461	38	85	0.70	6,1	9,0	10.90	111	
6/15	1165	1265	645	620	49	92	0.66	7,0	10.4	10.90	116	
6/22	1385	1281	615	666	52	108	0.68	7,9	9,8	10.00	128	
6/29	946	1186	700	484	41	80	0.67	6,0	14,6	9.80	121	No bile pigment increase
7/6	1100	1038	571	468	45	106	0.73	7,3	15,0	10.00	103	
7/13	1138	1138	683	455	40	100	0.86	5,8	14,4	9.90	115	
7/18	1120	1288	824	464	36	87	0.84	5,2	9,8	10.30	125	No bile pigment increase
7/19	Diet: Mixed diet											
8/9	Death from bile fistula intoxication											

Bile pigment daily output per 6 hours before this experiment (30 day average) = 21.8 mgm.

Bile pigment daily output per 6 hours during this experiment (30 day average) = 15.2 mgm.

No previous anemia experiments on this dog.

Blood volume with dry oxalate. Hemoglobin by Sahli tubes.

We may recall that the fasting bile fistula dog will react as promptly to anemia as the normal dog (table 23) and the bile fistula dog on a bread and milk diet also presents a normal blood regeneration curve

(table 32). But the meat diet reaction in the bile fistula dog is not like the reaction of the normal animal. The unfavorable clinical reaction of the bile fistula dog to the meat diet is often conspicuous

TABLE 60

Blood regeneration—liver. Dog 19–83. Bull mongrel pup male

DATE, 1918-1919	PIGMENT VOLUME = Hb. PER CENT TIMES BLOOD VOLUME	BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	R. B. C. HEMATOCRIT	Hb.	COLOR INDEX	R. B. C. MILLION	W. B. C.	WEIGHT	BLOOD PER KILOGRAM	REMARKS
		cc.	*cc.*	*cc.*	*per cent*	*per cent*				*kgm.*	*cc.*	
12/2/18	1090	1001	540	466	46.1	108	0.59	9.1	8,8	9.30	109	R. B. C. small
12/2	Diet: Bread and milk											
12/3	Bled 250 cc.											
12/4	Bled 250 cc.											
12/6	489	790	586	202	25.5	62	0.63	4,9	13,8	8.80	90	* Slight
12/7	Bled 200 cc.											
12/9	630	1032	770	258	25.0	61	1.20		13.7	8.65	119	
12/9	Diet: 550 grams cooked beef liver† —100 calories per kilo											
12/16	873	910	536	370	40.6	96	0.94	5,1	11.4	8.85	103	
12/23	962	962	550	407	42.3	100	0.56	8,9	19,2	9.35	103	
12/30	1304	1100	566	530	48.1	118				9.90	111	
12/30	Diet: Mixed diet											
1/8/19	1300	1235	642	580	47.0	105	0.80	6,6	14,2	10.75	115	
1/15	1095	1200	670	519	43.2	91	0.75	6,1	12,8	11.40	105	
1/22	1355	1300	685	600	46.2	104	0.75	6,9	8,0	11.30	115	

*Poikilocytosis of red blood cells.

† Beef liver cooked, fat and connective tissue removed, ground.

No previous anemia experiments on this dog.

(diarrhea and loss of weight and activity) and this may explain the observations recorded in tables 58 and 59.

Tables 60 and 61 show the remarkable influence which cooked liver exerts upon blood regeneration. As the sole article of food cooked liver may not be well tolerated, but in these two experiments the dogs

ate all of the liver and gained weight. The remarkable gain in hemoglobin, red cells and pigment volume is at once obvious at a glance. One experiment (table 61) shows practically complete regeneration in 2 weeks from the usual anemia level and both dogs are more than

TABLE 61

Blood regeneration—liver. Dog 18–114. Bull mongrel, female, young adult

DATE, 1918	PIGMENT VOLUME = Hb. PER CENT TIMES BLOOD VOLUME	BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	R. B. C. HEMATOCRIT	Hb.	COLOR INDEX	R. B. C. MILLION	W. B. C.	WEIGHT	BLOOD PER KILOGRAM
		cc.	*cc.*	*cc.*	*per cent*	*per cent*				*kgm.*	*cc.*
11/14	1640	1411	643	763	54.0	116	0.58	11.3	14.8	13.50	105
11/14	Diet: Crackermeal and milk										
11/15	Bled 353 cc.										
11/16	Bled 353 cc. No distress										
11/18	852	1077	695	372	34.5	79				12.85	84
11/18	Bled 270 cc. No distress										
11/20	592	1015	724	280	27.6	58	0.82	3,5	14,2	12.50	81
11/20	Diet: 750 grams cooked beef liver*										
11/27	1305	1290	726	540	41.8	101	0.74	6,8	11,8	13.25	97
12/4	1730	1480	740	720	48.5	117	0.76	7,7	20,0	13.35	111
12/11	1902	1516	728	766	50.5	125	0.82	7,6	13,4	13.65	111
12/13	Diet: Mixed diet										
12/20	1479	1333	713	613	46.0	111	0.65	8,6	14,6	13.90	96
12/27	1295	1276	670	594	46.5	101				13.75	93
1/10/19	1625	1390	668	716	51.5	117	0.68	8,6	12,8	14.20	98

* Beef liver cooked, fat and connective tissue removed, ground.
Experimental history, see table 12-b.

back to normal in 3 weeks. We are able to refer to a number of other experiments on this dog (table 61, dog 18-114, exper. history table 12-b) to give a good line on the type normal blood regeneration.

Table 62 shows another experiment with cooked beef liver but the amount of liver fed is much less and the rest of the food caloric value

is made up by crackermeal and milk. We see this small amount of liver causing a prompt rise of the hemoglobin and red cells to normal in 3 weeks, whereas the crackermeal and milk alone would require at least 5 to 6 weeks for complete regeneration.

TABLE 62

Blood regeneration—liver and crackermeal. Dog 19–15. Brindle bull mongrel, female, young adult

DATE, 1918	PIGMENT VOLUME = Hb. PER CENT TIMES BLOOD VOLUME	BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	R. B. C. HEMATOCRIT	Hb.	COLOR INDEX	R. B. C. MILLION	W. B. C.	WEIGHT	BLOOD PER KILOGRAM	REMARKS
		cc.	*cc.*	*cc.*	*per cent*	*per cent*				*kgm.*	*cc.*	
8/9	1702	1120	462	650	58.0	152	0.95	7.9	6,2	10.85	103	
8/9	Diet: Crackermeal and milk											
8/12	Bled 280 cc. No distress											
8/13	Bled 280 cc. No distress											
8/15	663	762	501	253	33.2	87				10.40	73	
8/15	Bled 190 cc. No distress											
8/17	468	669	493	173	25.8	70	0.92	3.8	16,8	10.35	65	* Poik. ++
8/17	Diet: 70 grams cooked beef liver,† 200 grams crackermeal, 500 cc. milk											
8/23	664	874	517	267	30.5	76	0.88	4.3	15,0	10.85	81	*
8/30	1290	949	466	478	50.3	136	0.90	7.5	15,8	11.30	84	
9/6	1600	1096	504	588	53.6	146	0.92	7.9	8,2	11.20	98	
9/13	1700	1089	495	594	54.5	156	0.96	8.1	10,8	11.50	95	

* Poikilocytosis of red blood cells.

† Beef liver cooked, fat and connective tissue removed, ground.

No previous anemia experiments on this dog. See table 64 for subsequent experiment.

Table 63 is not very satisfactory but is included because several interesting points may be made. This dog at the beginning of the experiment was very young, approximately 4 months, but the date of birth was not positively known. The pup had a hemoglobin of 73 per cent, which is not unusual in young dogs of this age. The amounts bled were less than normal because of this fact, but the total reserve

was evidently considerable, as indicated by the high figures after the bleeding. The young dog was growing rapidly during the whole period of the experiment, a gain of 3 kilos body weight in 6 weeks. The blood volume shows a considerable increase during this period, almost 100 per cent gain. The gain in hemoglobin and hematocrit is steady and

TABLE 63

Blood regeneration—liver and bread. Dog 18–114. Bull mongrel pup, female

DATE, 1918	PIGMENT VOLUME = Hb. PER CENT TIMES BLOOD VOLUME	BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	R. B. C. HEMATOCRIT	Hb.	COLOR INDEX	R. B. C. MILLION	W. B. C.	WEIGHT	BLOOD PER KILOGRAM
		cc.	*cc.*	*cc.*	*per cent*	*per cent*				*kgm.*	*cc.*
4/24	553	758	455	291	38.4	73	0.53	6,9	23,6	7.85	94
4/24	Diet: Bread and milk										
4/26	Bled 189 cc.										
4/27	Bled 90 cc. No distress										
4/29	435	805	528	255	31.7	54	0.58	4,7	14,6	7.65	105
4/30	Diet: 200 grams bread, 500 cc. milk, 50 grams cooked beef liver,* ground up with bread										
5/8	599	740	432	293	39.6	81	0.56	7,2	8,4	8.00	93
5/15					43.6	88	0.52	8,5	20,6	8.80	
5/22	825	938	514	411	43.8	87	0.54	8,0	16,0	9.50	98
5/29	791	807	463	330	40.9	98	0.69	7,1	22,8	9.60	84
5/29	Diet: Mixed diet										
6/5		902	497	391	43.4					10.45	86
6/10	1059	1009	556	443	43.9	105	0.67	7,8	11,8	10.70	94

* Beef liver cooked, fat and connective tissue removed, and ground up with bread.

Experimental history, see table 12–b.

No previous anemia experiments on this dog.

comes close to the average normal at the end of the experiment, in marked contrast to the level at the beginning of the experiment.

Meat extract (tables 64 and 65) evidently does not add anything to a given diet which in itself is especially favorable to blood regeneration. We have no evidence to show that meat extract is favorable or unfa-

vorable to blood reconstruction. Table 64 shows the rapid blood regeneration which we expect on a cooked liver diet and the return to normal requires but 3 weeks. We note again in this experiment the fall which often occurs following a sudden change to another diet

TABLE 64

Blood regeneration—liver and beef extract. Dog 19–15. Brindle bull mongrel, female, young adult

DATE, 1918	PIGMENT VOLUME = Hb. PER CENT TIMES BLOOD VOLUME	BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	R. B. C. HEMATOCRIT	Hb.	COLOR INDEX	R. B. C. MILLION	W. B. C.	WEIGHT	BLOOD PER KILOGRAM
		cc.	*cc.*	*cc.*	*per cent*	*per cent*				*kgm.*	*cc.*
11/14	1615	1260	506	746	59.3	128	0.76	8,4	10,8	12.40	102
11/14	Diet: Crackermeal and milk										
11/15	Bled 315 cc. No distress										
11/16	Bled 315 cc. No distress										
11/18	584	808	550	255	31.5	72				11.95	68
11/18	Bled 202 cc.										
11/20	508	796	568	224	28.1	64	0.69	4,6	14,4	11.35	70
11/20	Diet: 450 grams cooked beef liver* and 10 grams Liebig's beef extract										
11/27	808	917	565	347	37.9	88	0.76	5,8	12,8	11.30	81
12/4	1100	1002	555	458	45.0	108	0.66	8,2	15,3	11.50	89
12/11	1300	1084	560	518	47.8	120	0.60	10,3	12,2	11.35	95
12/13	Diet: Mixed diet										
12/20	912	954	550	395	41.6	96	0.56	8,6	16,8	11.95	80
12/27	926	1010	564	427	42.2	92				11.85	85
1/10/19	1122	1060	540	508	48.0	106	0.91	8,0	13,6	12.00	88

* Beef liver cooked, fat and connective tissue removed, ground.

Refer to table 62 for previous experiment.

(mixed diet) which, too, is very favorable for blood regeneration and maintenance. It is clear that there is no fluctuation in plasma volume which would supply an easy explanation for this phenomenon.

Meat extract (table 65) does not modify the reaction which may be expected following a liberal bread and milk diet. The blood picture is

returned almost to normal in 4 weeks after the anemia and there is a gain in weight of 1 kilo. A change to a mixed diet gives a favorable reaction as is usual under these circumstances. We may refer also to a part of table 66, which gives more data on the influence of bread and

TABLE 65

Blood regeneration—meat extract—bread and milk. Dog 18–116. Bull mongrel pup, female

DATE, 1918	PIGMENT VOLUME = Hb. PER CENT TIMES BLOOD VOLUME	BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	R. B. C. HEMATOCRIT	Hb.	COLOR INDEX	R. B. C. MILLION	W. B. C.	WEIGHT	BLOOD PER KILOGRAM	REMARKS
		cc.	*cc.*	*cc.*	*per cent*	*per cent*				*kgm.*	*cc.*	
4/24	903	962	459	486	50.5	94	0.65	7.2	9,8	9.75	99	* Slight
4/24	Diet: Bread and milk											
4/25	Bled 240 cc. from jugular vein											
4/26	Bled 190 cc. from jugular vein											
4/29	422	862	594	259	30.0	49	0.57	4,3	10.2	9.65	89	* Slight
4/29	Bled 110 cc. from jugular vein											
4/30	Diet: 200 grams bread, 500 cc. milk, 10 grams Liebig's beef extract											
5/8	717	956	591	347	36.3	75	0.48	7,8	10,0	9.60	99	*
5/15	882	1297	760	517	39.9	68	0.46	7,3	8,6	9.50	137	*
5/22	859	1035	594	430	41.5	83	0.51	8,2	22,8	10.70	97	*
5/29	865	961	550	403	41.8	90	0.54	8,3	10,2	10.85	89	*
5/29	Diet: Mixed diet											
6/10	1207	1128	634	483	42.8	107	0.63	8,5	30,0	12.50	90	
6/18						104						

* Poikilocytosis of red blood cells.

Experimental history, see table 13–b.

milk plus meat extract. The meat extract adds nothing to the reaction which is identical with the expected reaction from the bread and milk alone.

Tables 66 and 67 are to be considered together. The experiments were done at the same time under identical conditions and the results

TABLE 66

Blood regeneration—watery liver extract and sugar; bread and milk; meat extract, bread and milk; beef heart, bread and milk. Dog 17–157. Coach mongrel, female, young adult

DATE, 1919	PIGMENT VOLUME = Hb. PER CENT TIMES BLOOD VOLUME	BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	R. B. C. HEMATOCRIT	Hb.	COLOR INDEX	R. B. C. MILLION	W. B. C.	WEIGHT	BLOOD PER KILOGRAM	REMARKS
		cc.	*cc.*	*cc.*	*per cent*	*per cent*				*kgm.*	*cc.*	
2/20	1340	1180	567	606	51.4	113	0.79	7.2	8,2	10.35	114	
2/20	Diet: Bread and milk											
2/21	Bled 295 cc. No distress											
2/22	Bled 295 cc. Distress. Injected 50 cc. of 5 per cent sugar solution											
2/24	420	806	602	198	24.6	52	0.96	2,7	8,6	9.50	85	
2/26	Diet: 100 grams sugar, 10 grams watery liver extract,† 250 cc. water											
3/5	720	868	519	345	39.7	83	0.90	4,6	11,0	8.95	97	
3/12	700	813	493	312	38.4	86	0.88	4,9	7,2	8.25	99	
3/18	666	730	420	294	40.2	91	0.80	5,7	8,8	8.05	91	
3/18	Diet: 200 grams bread, 300 cc. milk											
3/26	824	929	560	360	38.7	89	0.85	5,2	10,0	8.40	110	
3/26	Diet: 200 grams bread, 300 cc. milk, 10 grams commercial meat extract											
4/2	756	913	556	398	38.1	83	0.90	4,6	10,8	8.75	104	
4/8	635	829	522	298	36.0	77	0.67	5,7	14,0	8.75	95	*
4/14	714	876	538	324	37.0	82	0.73	5,6	11,2	8.75	100	
4/21	860	984	584	389	39.6	87	0.72	6,0	12,8	8.90	110	
4/21	Diet: 200 grams beef heart (cooked), 200 grams bread, 300 cc. milk											
4/28	772	942	588	345	36.6	82	0.67	6,1	12,4	9.55	99	
5/7	842	961	572	384	40.0	88	0.73	6,0	15,4	10.00	96	
5/12	890	998	590	397	39.8	89	0.72	6,2	15,0	9.90	101	
5/12	Diet: Mixed diet											

* Poikilocytosis of red blood cells.

† Watery liver extract: Beef liver cut up into small cubes, allowed to stand in water over night in ice-chest, boiled in same water, and filtered. Filtrate concentrated to thick paste.

TABLE 66–B

Experimental history. Dog 17–157

EXPERIMENT NUMBER	DIET	BLOOD REGENERATION		WEIGHT	REMARKS
		Pigment volume	Blood per kilogram		
				kgm.	
Begin 9/3/17	Crackermeal, lard and gelatin	1025	115	8.40	
Bled 496 cc.		380	83	8.20	
End 10/25/17		890	102	8.70	
Begin 3/6/18	Crackermeal, lard, butter and Blaud's pills	1084	99	9.80	Table 71
Bled 488 cc.		614	90	9.10	
End 4/9/18		984	106	8.30	
Begin 5/20/18	Sugar, metabolism, desiccated beef heart	1113	120	10.20	
Bled 612 cc.		313	75	9.50	
End 6/18/18		663	100	7.60	Maximum regeneration 3 weeks. Pigment volume 698 cc.
Begin 8/28/18	Hb. and sugar feeding	1366	102	10.10	Table 78
Bled 710 cc.		433	79	9.25	(3 bleedings)
End 11/12/18	Hb. intravenously and sugar feeding Hb. intravenously and crackermeal + milk Crackermeal, milk and dried yeast	886	114	8.15	Pigment volume 805 at end of Hb. period
Begin 2/20/19	Liver extract, sugar	1340	114	10.35	Table 66
Bled 590 cc.		420	85	9.50	
End 5/12/19	Bread, milk, meat extract Beef heart, bread and milk	890	101	9.90	Pigment volume 666 cc. at end of sugar feeding

are very suggestive. Both dogs have been observed in many other experiments and their anemia reactions are therefore well known. One dog was given the watery extract of beef liver and the other the liver residue. Sugar, 100 grams, was added to each feeding and it is clear

TABLE 67

Blood regeneration—liver residue and sugar; bread and milk; crackermeal and bread and milk; cooked beef liver and bread and milk. Dog 16–158. Coach mongrel, male, young adult

DATE, 1919	PIGMENT VOLUME = Hb. PER CENT TIMES BLOOD VOLUME	BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	R. B. C. HEMATOCRIT	Hb.	COLOR INDEX	R. B. C. MILLION	W. B. C.	WEIGHT	BLOOD PER KILOGRAM	REMARKS
		cc.	*cc.*	*cc.*	*per cent*	*per cent*				*kgm.*	*cc.*	
2/20	1862	1375	549	820	59.6	135	0.77	8,8	7,0	12.35	111	
2/20	Diet: Bread and milk											
2/21	Bled 344 cc. No distress											
2/22	Bled 344 cc. Dyspnea; injected 50 cc. of 5 per cent sugar solution											
2/24	823	983	625	353	35.9	84	1.02	4,1	19,6	11.40	86	
2/25	Bled 246 cc.											
2/27	478	778	562	211	27.2	61	0.85	3,6	8,4	11.10	70	
2/27	Diet: 100 grams sugar, 200 cc. water by stomach tube; 100 grams liver residue†											
3/5	692	867	531	331	38.2	80	0.76	5,3	6,4	10.75	81	*
3/12	876	900	517	373	41.5	97	0.84	5,8	5,4	9.95	90	* Poik.+
3/18	738	754	421	329	43.7	98	0.83	5,9	14,4	9.15	82	* Poik.+
3/18	Diet: 200 grams bread, 300 cc. milk											
3/26	725	923	597	316	34.3	79	0.77	5,1	6,8	10.15	91	* Poik.
3/26	Diet: 200 grams bread, 100 grams crackermeal, 300 cc. milk											
4/2	748	944	600	339	35.9	79	0.61	6,5	9,8	10.50	90	* Poik.+
4/8	685	966	616	340	35.2	71	0.61	5,8	10,8	11.10	87	* Poik.
4/14	762	968	608	355	36.7	79	0.59	6,7	7,8	12.75	76	* Poik.
4/21	840	1072	640	406	37.9	78	0.56	7,0	10,6	11.20	96	
4/21	Diet: 200 grams cooked beef liver, 200 grams bread, 300 cc. milk											
4/28	820	1027	629	388	37.8	80	0.73	5,5	12,0	11.95	86	
5/7	1155	1155	595	549	47 5	100	0.75	6,7	9,6	11.45	101	
5/12	1074	1095	583	502	45.8	98	0.66	7,4	13,0	11.80	93	* Poik.+
5/12	Diet: Mixed diet											

* Poikilocytosis of red blood cells.

† Liver residue: Residue left after water and alcoholic extraction; put in meat press and all liquid removed. Just before feeding residue was again washed and brought to boiling point to remove all traces of alcohol. Dog did not always eat full amount of food mixture.

Experimental history, see table 46–b.

that both the liver watery extract and liver residue exert a certain influence upon the blood regeneration which is much more than can be accounted for by the sugar alone. The liver residue has greater influence upon the blood regeneration than does the liver watery extract but the difference is not striking.

Bread and milk feeding for 1 week subsequent to these sugar and liver periods does not cause much reaction. There is a slight loss in hemoglobin and red cell hematocrit in experiment 67 (liver residue).

The next 4 weeks are similar and bread or crackermeal and milk are the essential features of the diet. The level of pigment volume, red cell hematocrit and hemoglobin is constant. There is slight gain in the red cell counts.

We have pointed out the fact that a prolonged diet period unfavorable to hemoglobin regeneration will leave the dog in a condition which may be clinically excellent but from the standpoint of formation of hemoglobin very unfavorable. These two dogs were still anemic although in excellent physical condition and of practically normal weight. Under such circumstances we feel that any diet is given its most severe test and few diets of limited nature can give a favorable reaction as regards blood regeneration. Under ordinary circumstances a diet of beef heart, bread and milk (table 66) is favorable for blood regeneration but under this severe test with unfavorable conditions we note little if any blood regeneration during a period of 3 weeks.

Cooked beef liver with bread and milk even under these same unfavorable conditions is able to effect a prompt blood regeneration—conspicuous in red cell hematocrit, red count and hemoglobin. This is the severest test of any diet factor in its relation to blood regeneration. Also refer to table 22 (same experiment).

DISCUSSION

The reader will observe many individual differences in the reactions of various dogs to a unit type of secondary anemia. Some of these vagaries we are as yet unable to explain but others are now much clearer than in the earlier stages of this investigation. We have noted that on occasions certain dogs *made anemic* for the *first time* presented a most unusually rapid regeneration, even on a very limited diet. This is not the rule, but is sufficiently common to suggest caution in conclusions drawn from such experiments. A repeat experiment will

give the true constant reaction. How to explain this fact is not clear to us, but a simple way out is to assume a reserve present in the body under these conditions which permit of unusual blood regeneration even under most unfavorable diet conditions. A knowledge of this fact may keep the investigator from falling into error in deductions drawn from single experiments. With this exception the dogs will show uniform reactions when we repeat anemia experiments under uniform conditions.

It is clear that long limited diet periods following the standard anemia may preserve the dogs in excellent physical condition as concerns weight, general condition and activity. These same dogs, however, may continue to present a definite anemia. Under such conditions many diet mixtures are unable to stimulate any blood regeneration. Any diet factors are put to the severest test when they are administered to dogs under such circumstances and we are inclined to accept this as the severest test for any given diet factor. Cooked liver gives a very favorable reaction and causes blood regeneration even under these severe test conditions.

Following a simple anemia many diet mixtures, if given at once, will cause a distinct gain in hemoglobin and red cells. But if a limited diet period intervenes between the anemia and the exhibition of the test diet we will see a negative reaction. This may be illustrated by bread and milk given in *liberal amounts* sufficient to permit a gain in body weight or at least a maintenance of body weight. If the dog is bled and at once placed on a bread and milk diet there will usually appear a slow steady gain in pigment volume. If the same dog is placed on a limited *carbohydrate diet* for 3 to 4 weeks before being changed to a liberal bread and milk diet we will usually observe subsequently an unchanged level of pigment volume, red cell hematocrit and hemoglobin. Under such unfavorable conditions the bread and milk diet is unable to give a favorable reaction for the blood regeneration. In certain experiments the cooked beef heart is also unable to modify the curve of blood regeneration under these unfavorable conditions. Cooked liver is able to induce blood regeneration even under the most unfavorable conditions. The same is true for the common *mixed diet* of table scraps. It is important to keep these facts in mind when we evaluate the reaction following the administration of a given diet factor under different experimental conditions.

The question at once confronts us: what part of the meat or liver substance is responsible for the favorable blood reaction? First we

must investigate the pigment substances present in the meat—for example, the hemoglobin and myohematin. Some experiments with hemoglobin appear in the next paper of this series, and we hope soon to report other experiments dealing with the myohematin pigment.

SUMMARY

Cooked lean beef and beef heart are diet factors of importance as regards blood regeneration subsequent to simple secondary anemia. These food substances alone or in combination with other foods will give a rapid blood regeneration after anemia.

Anemia is produced by bleeding one-fourth of the measured blood volume on each of 2 successive days. This anemia will be completely repaired within 3 to 4 weeks if the dog is given a liberal diet of meat or beef heart.

Cooked liver is as sufficient as meat and may be even more efficient in promoting complete blood regeneration subsequent to a standard anemia. Blood regeneration may be completed in 2 to 4 weeks.

Commercial meat extract is inert and watery liver extract has but little influence upon blood regeneration.

The meat diet reaction in the bile fistula dog is not exactly like the reaction of the normal animal.

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BLOOD REGENERATION FOLLOWING SIMPLE ANEMIA

V. The Influence of Blaud's Pills and Hemoglobin

C. W. HOOPER, F. S. ROBSCHEIT AND G. H. WHIPPLE

From the George Williams Hooper Foundation for Medical Research, University of California Medical School, San Francisco

Received for publication April 3, 1920

The familiar fact that iron in some form is very frequently given in cases of secondary anemia made it imperative to test this drug under a variety of experimental conditions. The outstanding fact in our experiments is that *iron given as Blaud's pills has no influence in secondary anemia under the conditions of these experiments.* It may be objected that the Blaud's pills were not fresh or were not dissolved in the dog's intestinal tract. We obtained these Blaud's pills from a large wholesale firm in this city and they were soft and easily crushed into a soft, pasty material. The pills were crushed before being given by mouth. Further objections may be made that this drug has no influence on the dog but does have potency when administered to human beings. This of course is not subject to proof, but the claims for the potency of iron in conditions of secondary anemia do not stand on firm ground. We invite attention to the profound influence which is properly attributed to diet factors. Those who claim that iron is a potent drug must exclude the food factors which are known to be concerned before they can prefer too many objections to our negative results.

The feeding of blood has at times been used in the treatment of secondary anemia. We are able to find some experimental evidence to support this treatment, but whole red cells or hemoglobin given by mouth in the form of a dry powder do not appear to influence profoundly the blood regeneration curve. Our experiments show that hemoglobin does have a distinct influence on blood regeneration but not sufficient to warrant its use in uncomplicated secondary anemia in view of the favorable reactions due to meat and other diet factors.

The favorable reaction which seems to accompany administration of hemoglobin by injection (intravenous and intraperitoneal) may be

of some value in the treatment of certain forms of anemia. It is possible that the reaction to this type of injection may differ from that associated with a transfusion and in certain diseases this procedure (hemoblobin injection) may stimulate rather than depress the bone marrow. Further experimental work is in progress.

EXPERIMENTAL OBSERVATIONS

The same technical procedures are used in these experiments as have been described in the first paper of this series. Blaud's pills were given daily. The experimental histories give the complete list of anemia experiments on any given dog and are referred to in each experiment. The control experiments are frequently given in the other papers of this series, but the proper reference is appended to the experiment dealing with iron or hemoglobin. We hope to report experiments in the near future dealing with other drugs used in the treatment of anemia. We expect to test many other pigment substances besides hemoglobin as to their influence on blood regeneration. This includes animal and vegetable pigments as they occur in various meats, fish and vegetables.

Table 68 represents a long anemia experiment with Blaud's pills which is conclusive in showing the complete failure of this drug (carbonate of iron) to influence blood regeneration under the conditions of the experiment. Subsequent experiments will make it clear that *Blaud's pills are inert* in so far as any influence on this type of anemia is concerned.

The experimental history of this dog (table 18–b) gives the reaction of this animal to other diet factors and establishes the type reaction to secondary anemia. It is fair to say that this same type of blood regeneration would be expected without the influence of Blaud's pills. This dog is more resistant than usual and tolerated this limited diet for a long period without any symptoms of dietary deficiency disease. The falling hemoglobin, red cell count and weight curves, however, may indicate an impending dietary deficiency complex which did not develop because of the change to a liberal mixed diet.

The Blaud's pills were given daily and crushed before administration by mouth. Sufficient bread and skim milk were given to maintain the body weight constant until the last 2 weeks. During the first 8 weeks the red cell hematocrit is stationary but the hemoglobin rises slowly. During this period the red count rises slowly and uniformly

TABLE 68

Blood regeneration—bread, milk and Blaud's pills. Dog 16–169. Bull mongrel, female, young adult

DATE, 1917	PIGMENT VOLUME = Hb. PER CENT TIMES BLOOD VOLUME	BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	R. B. C. HEMATOCRIT	Hb.	COLOR INDEX	R. B. C. MILLION	W. B. C.	WEIGHT	BLOOD PER KILOGRAM	REMARKS
		cc.	*cc.*	*cc.*	*per cent*	*per cent*				*kgm.*	*cc.*	
2/12	939	978	489	489	50	96	0.73	6,6	6,2	7.70	127	
2/12	Diet: Bread and milk											
2/13	Bled 244 cc.											
2/14	Bled 244 cc.											
2/15	267	580	423	157	27	46	0.77	3,0	6,8	7.40	80	
2/15	Bread, milk and 2 Blaud's pills daily											
2/21	348	809	566	243	30	43	0.86	2,5	10,8	7.50	108	
2/28	375	694	493	201	29	54	0.71	3,8	7,4	7.40	94	
3/7	393	667	487	180	27	59	0.69	4,3	5,6	7.30	91	*
3/14	428	738	524	214	29	58	0.66	4,4	11,0	7.20	104	*
3/21	335	697	495	202	29	48	0.52	4,6	7,4	6.80	103	*
3/28	312	625	431	194	31	50	0.48	5,2	10,0	7.00	89	*
4/5	382	694	486	208	30	55	0.48	5,7	6,8	6.80	102	*
4/11	368	681	470	211	31	54	0.46	5,9	13,6	7.00	97	*
4/18	500	725	471	254	35	69	0.51	6,7	11,2	7.30	99	*
4/25	576	728	480	258	35	78	0.51	7,7	5,6	7.20	102	*
5/4	560	700	455	245	35	80	0.52	7,7	8,6	7.00	100	*
5/11	617	771	455	316	41	80	0.53	7,6	12.4	7.40	104	*
5/18	624	762	457	305	40	82	0.55	7,4	9.4	7.20	106	*
5/25	688	819	467	352	43	84	0.57	7,3	6,6	7.00	117	*
6/1	716	721	418	303	42	99	0.59	8,4	6,6	6.80	106	*
6/8	830	847	491	355	42	98	0.64	7,6	5,4	7.00	121	*
6/15	833	833	500	333	40	100	0.66	7,6	5,8	6.50	127	*
6/27	705	839	520	319	38	84	0.62	6,8	10,0	6.50	129	*
7/11	477	597	394	203	34	80	0.67	6,0	7,6	6.20	96	*
7/16	456	570	393	177	31	80	0.74	5,4	6,2	5.90	97	*

* Marked fragmentation of red blood cells.
Blood volume with dry oxalate. Hemoglobin by Sahli tubes.
Experimental history, see table 18–b.

and continues this rise to a figure even above normal. It is noted, however, that there was marked fragmentation of the red cells and we can scarcely account for this great increase in red cells (2,500,000 to 8,400,000) with the red cell hematocrit showing only a rise from 27 per cent to 42 per cent, except on the ground of red cell fragmentation or abortive red cell construction. The hemoglobin does not keep pace

TABLE 69

Blood regeneration—bread, milk and Blaud's pills. Dog 17–193. Bull mongrel, female, young adult

DATE, 1917	PIGMENT VOLUME = Hb. PER CENT TIMES BLOOD VOLUME	BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	R. B. C. HEMATOCRIT	Hb.	COLOR INDEX	R. B. C. MILLION	W. B. C.	WEIGHT	BLOOD PER KILOGRAM	REMARKS
		cc.	*cc.*	*cc.*	*per cent*	*per cent*				*kgm.*	*cc.*	
4/24	1089	1100	517	584	53	99	0.75	6.6	15,8	8.40	131	
4/24	Diet: Bread and milk											
4/25	Bled 275 cc.											
4/26	Bled 275 cc.											
4/27	333	710	547	163	23	47	0.87	2,7	24,0	8.20	87	
4/27	Diet: Bread, milk and 2 Blaud's pills daily											
5/2	458	683	492	191	28	67	0.82	4,1	9,4	8.00	85	
5/9	636	795	493	302	38	80	0.75	5,3	9,4	8.00	99	
5/16	856	1006	533	473	47	85	0.76	5,6	6,6	8.00	102	
5/23	1008	1039	561	478	46	97	0.69	7,0	7,4	8.00	129	*
5/30	1041	1021	521	500	49	102	0.67	7,6	12,4	8.20	124	*
6/6	1055	1014	527	487	48	104	0.65	8,0	6,2	7.90	128	*

* Fragmentation of red blood cells.

No previous anemia experiments on this dog.

Blood volume with dry oxalate. Hemoglobin by Sahli tubes.

with the red count and a fall in the color index from 77 per cent to 46 per cent is recorded.

The high water mark for blood regeneration is noted after $3\frac{1}{2}$ months and the level at this time is far from normal. Subsequently there is a loss in red cell hematocrit, red count, hemoglobin and pigment volume. We believe this indicated a tendency toward a dietary deficiency dis-

ease which would have developed had the bread and milk diet been continued. The plasma volume as usual is constant throughout the entire experiment with the exception of the last 2 weeks.

The next experiment (table 69) shows a fairly complete blood regeneration during a period of 5 weeks on a bread and milk diet plus Blaud's pills. It is to be observed that no previous anemia experi-

TABLE 70

Blood regeneration—crackermeal, lard, butter, milk powder and Blaud's pills. Dog 17–205. Bull mongrel, male, young adult

DATE, 1918	PIGMENT VOLUME = Hb. PER CENT TIMES BLOOD VOLUME	BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	R. B. C. HEMATOCRIT	Hb.	COLOR INDEX	R. B. C. MILLION	W. B. C.	WEIGHT	BLOOD PER KILOGRAM	REMARKS
		cc.	*cc.*	*cc.*	*per cent*	*per cent*				*kgm.*	*cc.*	
3/4	1182	1055	538	518	49	112	0.70	8,0	10,8	12.60	84	
3/4	Diet: 279 grams crackermeal, 10 grams lard, 10 grams butter											
3/6	Bled 274 cc.											
3/7	Bled 254 cc.											
3/9	605	840	605	235	28	72	0.82	4,4	30,4	12.1	69	*Anis.
3/9	Diet: 142 grams crackermeal, 10 grams lard, 10 grams butter, 279 grams milk powder, 2 Blaud's pills daily											
3/15	565	807	573	234	29	70	0.88	4,0	27,4	11.40	71	*Anis.
3/20	798	928	622	306	33	86	0.93	4,6	9,2	11.20	83	*Anis.
3/27	682	802	545	256	32	85	0.79	5,4	19,2	10.90	73	*Anis. + +
4/3	983	919	597	323	35	107	0.82	6,5	21,6	10.90	84	*Anis. + +
4/9	795	750	480	270	36	106	0.79	6,7	7,4	9.60	78	*Anis. + +
4/9	Dietary deficiency disease. Recovery.											

* Anisocytosis of red blood cells.

Refer to table 34 for control.

Blood volume with dry oxalate. Hemoglobin by Sahli tubes.

ments had been done on this dog and the capacity for blood regeneration may be greater under such circumstances. The reaction is not unusual, however, in view of the liberal amounts of bread and milk which were sufficient to maintain the body weight. It cannot be granted that the Blaud's pills had any influence upon the blood regeneration.

Table 70 is of considerable interest because we are able to refer to a control period (table 34) on a similar diet but without the Blaud's pills. If anything the control period shows slightly more blood regeneration during the first 5 weeks. The control experiment was of much longer duration (12 weeks) without dietary deficiency disease symptoms. This may be due to the fact that this control experiment (table 34)

TABLE 71

Blood regeneration—crackermeal, lard, butter and Blaud's pills. Dog 17–157. Coach mongrel, female, young adult

DATE, 1918	PIGMENT VOLUME = Hb. PER CENT TIMES BLOOD VOLUME	BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	R. B. C. HEMATOCRIT	Hb.	COLOR INDEX	R. B. C. MILLION	W. B. C.	WEIGHT	BLOOD PER KILOGRAM	REMARKS
		cc.	*cc.*	*cc.*	*per cent*	*per cent*				*kgm.*	*cc.*	
3/4	1084	976	537	439	45	111	0.82	6.8	8,4	9.80	99	
3/4	Diet: 199 grams crackermeal, 10 grams lard, 10 grams butter											
3/6	Bled 254 cc.											
3/7	Bled 234 cc.											
3/9	614	818	597	221	27	75	1.00	3,7	10,2	9.10	90	
3/9	Diet: 201 grams crackermeal, 10 grams lard, 10 grams butter, 2 Blaud's pills daily											
3/15	756	869	600	269	31	87	0.99	4,4	14,8	9.00	97	*
3/20	801	843	531	312	37	95	1.00	4,7	11,4	8.50	99	*
3/27	848	848	517	331	39	100	0.89	5,6	8,2	8.40	101	*
4/3	943	865	519	346	40	109	0.88	6,2	18,8	8.30	104	*
4/9	984	878	536	342	39	112	0.85	6,6	11,4	8.30	106	*

* Slight anisocytosis of red blood cells.

Experimental history, see table 66–b.

Blood volume with dry oxalate. Hemoglobin by Sahli tubes.

on crackermeal, lard and butter was the first anemia experiment done on this animal.

The control without Blaud's pills and this experiment (table 70) with Blaud's pills show practically identical anemia figures for red cell hematocrit and hemoglobin. The amount of red cell and hemoglobin regeneration is practically identical in the 5 weeks in the two experi-

ments. This again gives evidence that Blaud's pills are inert under these controlled conditions of experimental anemia and regeneration.

Table 71 shows a little more blood regeneration than usual but not enough to be able to attribute any of the reaction to the Blaud's pills. The red cell hematocrit reads 39 per cent at the end of 1 month and this is not a normal figure. The hemoglobin was low to start with

TABLE 72

Blood regeneration—bread, milk and Blaud's pills—splenectomy. Dog 17–142. Coach mongrel, female, age 4 to 5 months

DATE, 1917	PIGMENT VOLUME = Hb. PER CENT TIMES BLOOD VOLUME	BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	R. B. C. HEMATOCRIT	Hb.	COLOR INDEX	R. B. C. MILLION	W. B. C.	WEIGHT	BLOOD PER KILOGRAM
		cc.	*cc.*	*cc.*	*per cent*	*per cent*				*kgm.*	*cc.*
4/24	842	772	386	386	50	109	0.91	6,0	19,8	8.00	96
4/24	Diet: Bread and milk										
4/25	Bled 193 cc.										
4/26	Bled 193 cc.										
4/27	323	609	457	152	25	53	0.98	2,7	20,2	8.00	76
4/27	Diet: Bread, milk and 2 Blaud's pills daily										
5/2	390	600	390	210	35	65	0.90	3,6	18,4	7.80	76
5/9	760	835	451	384	46	91	0.83	5,5	13,6	7.90	106
5/16	837	790	411	379	48	106	0.80	6,6	7,2	7.70	103
5/23	997	906	453	453	50	110	0.75	7,3	6,4	7.70	117
5/30	890	832	416	416	50	107	0.70	7,7	11,4	8.00	104
6/6	950	863	440	423	49	110	0.73	7,5	11,6	7.70	112

No previous anemia experiments on this dog.
Blood volume with dry oxalate. Hemoglobin by Sahli tubes.

and returns to this level in 4 weeks. The type reaction of this dog is well established by the list of other anemia experiments given in table 66–b.

Table 72 shows a reaction to the first anemia experiment which has been noted in other experiments. It is not the rule but is frequent enough so that we must always be on our guard in discussing the *first* anemia experiment on any given dog. There may be this remarkable

reserve which enables the dog to give an unusual blood regeneration even on a most unfavorable diet. The blood regeneration is complete in 3 to 4 weeks and thereafter is maintained for the next 3 weeks at

TABLE 73

Blood regeneration—bread, milk and Blaud's pills—splenectomy. Dog 17–37. Bull mongrel, female, young adult

DATE, 1917	PIGMENT VOLUME = Hb. PER CENT TIMES BLOOD VOLUME	BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	R. B. C. HEMATOCRIT	Hb.	COLOR INDEX	R. B. C. MILLION	W. B. C.	WEIGHT	BLOOD PER KILOGRAM	REMARKS
		cc.	*cc.*	*cc.*	*per cent*	*per cent*				*kgm.*	*cc.*	
3/26	927	850	459	391	46	109	0.80	6,8	9,4	10.60	80	
3/29	Bled 212 cc. and 212 cc. (refer to table 8)											
4/23	Diet: Bread and milk following fasting experiment of 3 weeks' duration											
4/23	540	772	502	270	35	70	0.66	5,3	7,8	6.90	112	
4/30	578	802	521	281	35	72	0.67	5,4	11,6	8.20	98	
5/7	566	808	517	291	36	70	0.71	4,9	7,4	8.40	96	
5/14	662	808	517	291	36	82	0.68	6,0	6,8	8.40	96	*
5/21	656	830	531	299	36	79	0.69	5,7	5,6	8.90	93	
5/28	566	833	553	283	34	68	0.68	5,0	7,0	8.30	100	
6/4	524	759	530	228	30	69	0.73	4,7	5,2	8.60	88	
6/11	528	754	529	226	30	70	0.78	4,5	14,6	8.60	88	
6/11	Diet: Bread, milk and 2 Blaud's pills daily											
6/18	631	809	517	291	36	78	0.62	6,3	6,4	8.40	96	†
6/25	514	858	626	231	27	60	0.79	3,8	53,0	8.30	103	†
7/2	439	708	517	191	27	62	0.79	3,9	18,2	8.40	84	†
7/9	537	790	521	268	34	68	0.76	4,5	13,0	8.60	92	†
7/16	648	762	480	282	37	85	0.77	5,5	9,3	8.40	91	†

* Red cells are small and much fragmented.

† Red cells are large and fairly uniform in size.

Experimental history, see table 8–b.

Blood volume with dry oxalate. Hemoglobin by Sahli tubes.

the normal level. Refer to tables 74 and 75 for meat diet controls with no previous bleeding. The fact that the dog was very young (4 to 5 months) and was without a spleen is thought to be without influence on this general reaction noted in other dogs (adult and non-

splenectomized). We have no reason to suspect that the Blaud's pills were concerned in this reaction which has been noted in control experiments on the same diet without the Blaud's pills.

The second splenectomy experiment (table 73) shows a negative reaction with bread and milk alone as well as with bread and milk plus Blaud's pills. There are peculiar fluctuations during certain weeks

TABLE 74

Blood regeneration—meat and Blaud's pills. Dog 17–191. Bull mongrel, male, young adult

DATE, 1917	PIGMENT VOLUME = Hb. PER CENT TIMES BLOOD VOLUME	BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	R. B. C. HEMATOCRIT	Hb.	COLOR INDEX	R. B. C. MILLION	W. B. C.	WEIGHT	BLOOD PER KILOGRAM
		cc.	*cc.*	*cc.*	*per cent*	*per cent*				*kgm.*	*cc.*
4/24	1352	1206	567	640	53	112	0.71	7.9	13.4	9.50	127
4/24	Diet: Bread and milk										
4/25	Bled 301 cc.										
4/26	Bled 301 cc.										
4/27	382	780	600	187	24	49	0.79	3.1	17.6	9.30	84
4/27	Diet: Meat and 2 Blaud's pills daily										
5/2	536	800	543	264	33	67	0.91	3.7	17.8	9.50	84
5/9	1030	1064	585	479	45	97	0.90	5.4	8.2	9.50	112
5/16	1282	1256	653	604	48	102	0.73	7.0	11.8	9.80	128
5/23	1530	1377	647	730	53	111	0.66	8.4	8.8	9.70	142
5/30	1571	1366	615	752	55	115	0.65	8.8	18.6	10.00	136
6/6	1510	1314	591	419	55	115	0.66	8.7	7.2	9.60	137

No previous anemia experiments on this dog.
Blood volume with dry oxalate. Hemoglobin by Sahli tubes.

in both these periods and we believe these ups and downs are referable to the splenectomy. It is significant that the hemoglobin and red cell hematocrit changes are not associated with any constant change in plasma volume. The red count fluctuates with the pigment curve and we must assume periodic constructive or destructive waves influencing the red cells in the blood stream. From data already published (1) dealing with bile excretion in splenectomized dogs with simple

anemia we may suspect that blood destruction may be in part responsible for these irregularities in the level of the curves of pigment volume, red cell and hemoglobin values. The color index shows no change. There is a note to the effect that the red cells are larger and more uniform in size during the period of iron feeding. A single observation of this nature is of interest but does not call for discussion at this time.

TABLE 75

Blood regeneration—meat and Blaud's pills—splenectomy. Dog 17–163. Bull mongrel, male, young adult

DATE, 1917	PIGMENT VOLUME = Hb. PER CENT TIMES BLOOD VOLUME	BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	R. B. C. HEMATOCRIT	Hb.	COLOR INDEX	R. B. C. MILLION	W. B. C.	WEIGHT	BLOOD PER KILOGRAM
		cc.	*cc.*	*cc.*	*per cent*	*per cent*				*kgm.*	*cc.*
4/24	918	918	450	468	51	100	0.75	6,7	14,4	9.00	102
4/24	Diet: Bread and milk										
4/25	Bled 229 cc.										
4/26	Bled 229 cc.										
4/27	340	679	509	170	25	50	1.00	2,5	17,6	8.90	76
4/27	Diet: Meat and 2 Blaud's pills daily										
5/2	538	803	514	289	36	67	0.90	3,7	19,2	9.00	89
5/9	790	909	509	400	44	87	0.78	5,6	19,6	8.90	102
5/16	924	880	475	405	46	105	0.78	6,7	8,2	8.90	98
5/23	1140	1096	559	538	49	104	0.75	6,9	12,4	8.80	124
5/30	907	863	423	440	51	105	0.63	8,3	9,2	9.00	96
6/6	1080	1002	491	520	51	106	0.65	8,2	13,8	8.60	117

No previous anemia experiments on this dog.
Experimental history, see table 38–b.
Blood volume with dry oxalate. Hemoglobin by Sahli tubes.

The two meat feeding experiments (tables 74 and 75) give the expected reaction on this diet. The splenectomy dog reacts the same as does the normal dog. Sufficient meat is given to maintain a constant body weight. Both these dogs had not been used previously for anemia experiments; refer to table 72 which illustrates the reserve capacity sometimes exhibited by such dogs. We have no reason to suppose that the Blaud's pills were in any way concerned in this reaction.

Hemoglobin experiments.—Table 76 gives an experiment of much interest and we are able to submit the control experiment (table 16). This same dog a few months previously was made anemic and placed upon a sugar diet. After a period of 4 weeks the level of pigment volume, hemoglobin and red cell hematocrit was practically the same as that

TABLE 76

Blood regeneration—hemoglobin and sugar—metabolism. Dog 17-28. Bull mongrel, female, young adult

DATE, 1917	PIGMENT VOLUME = Hb. PER CENT TIMES BLOOD VOLUME	BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	R. B. C. HEMATOCRIT	Hb.	COLOR INDEX	R. B. C. MILLION	W. B. C.	WEIGHT	BLOOD PER KILOGRAM	REMARKS
		cc.	*cc.*	*cc.*	*per cent*	*per cent*				*kgm.*	*cc.*	
5/7	1895	1709	769	940	55	111	0.66	8,4	13,2	12.50	136	
5/7	Diet: Mixed food											
5/8	Bled 427 cc.											
5/9	Bled 427 cc.											
5/11	594	914	640	274	30	65	0.76	4,3	22,0	12.20	76	
5/11	Diet: 50 grams cane sugar, 25 grams glucose, 10 grams washed red blood cells											
5/14	558	979	676	303	31	57	0.75	3,8	11,2	11.50	85	
5/21	676	1055	665	390	37	64	0.70	4,6	6,8	10.80	98	*
5/28	856	1141	673	468	41	75	0.71	5,3	9,6	10.10	113	*
6/4	887	1137	603	534	47	78	0.58	6,7	9,6	9.50	119	*
6/11	920	1180	672	508	43	78	0.59	6,6	8,0	8.90	133	*
6/18	1085	1277	664	612	48	85	0.50	8,5	10,0	8.30	154	*

* Marked fragmentation of red cells.

400 cc. water given by stomach tube daily.

Experimental history, see table 6-b; see table 16 for control.

Blood volume with dry oxalate. Hemoglobin by Sahli tubes.

observed immediately after the bleeding. The total blood regeneration then in the control experiment was zero. There was a trifling gain in the red count.

Under identical conditions on a sugar diet plus 10 grams washed, dried red blood cells we see a very different reaction (table 76). The

TABLE 76-A

Total urinary nitrogen—hemoglobin and sugar. Dog 17–28

DATE, 1917	TOTAL NITROGEN 24 HOURS URINE	URINE TOTAL 24 HOURS	WEIGHT	REMARKS
	grams	*cc.*	*pounds*	
5/11	3.55	490	26.4	0 feces
5/12	3.19	480	25.8	Solid black stool
5/13		361	25.5	Solid black stool
5/14	3.50	386	25.3	0 feces
5/15	2.52	485	25.0	Slight diarrhea
5/16	2.58	358	24.7	Solid feces
5/17	2.38	390	24.5	0 feces
5/18	2.35	480	24.4	Trace of feces
5/19	2.30	403	24.1	Slight diarrhea
5/20	2.46	410	24.0	0 feces
5/21	2.46	420	23.8	0 feces
5/22	2.63	465	23.4	0 feces
5/23	2.41	383	23.4	0 feces
5/24	2.94	400	23.0	Diarrhea +
5/25	2.74	376	22.7	Diarrhea +
5/26	2.66	412	22.6	0 feces
5/27	2.52	395	22.4	0 feces
5/28	2.35	424	22.2	0 feces
5/29	2.46	370	21.9	0 feces
5/30	2.63	410	21.8	0 feces
5/31	2.13	510	21.6	0 feces
6/1	2.32	405	21.4	0 feces
6/2	2.18	405	21.4	0 feces
6/3	2.30	425	21.1	0 feces
6/4	2.24	426	20.8	
6/5	2.44	425	20.8	0 feces
6/6	3.00	417	20.5	Formed feces
6/7	2.24	373	20.2	Soft feces
6/8	2.46	410	20.0	Solid feces
6/9	2.58	419	19.9	0 feces
6/10	2.60	425	19.8	0 feces
6/11	2.63	407	19.5	0 feces
6/12	2.83	405	19.5	0 feces
6/13	2.83	375	19.4	0 feces
6/14	2.88	490	19.2	0 feces
6/15	3.00	480	18.9	0 feces
6/16	3.28	411	18.6	Solid feces
6/17	3.42	395	18.4	0 feces
6/18	3.02	400	18.3	

initial anemia level in the two experiments is practically identical, also the body weight, normal initial blood pigment, etc. The only point in which these two experiments differ lies in the 10 grams of red cells added to the sugar diet. At the end of 4 weeks the control shows a gain of zero in pigment substance but the red cell feeding gives a substantial gain of 13 per cent hemoglobin, 17 per cent red cell hematocrit and 300 units pigment volume. There is a gain of 2,400,000 in the red cell count. The subsequent 2 weeks show a distinct gain over the level just noted. This is in notable contrast to the expected reaction on a sugar diet.

The urinary nitrogen and fluid excretion figures are given (table 76–a) and show the expected values. During the last week of the experiment there is a distinct rise in the nitrogen output. We have come to look upon this as an early sign of intoxication which if not heeded may be followed by severe clinical disturbances and death. This dog promptly recovered when placed on a mixed diet.

It is to be noted that *whole red cells* were used in this experiment, that is, hemoglobin plus red cell stroma. In the following experiments hemoglobin alone was used. We might point out the difference in the color index observed in these two conditions but feel that the data are not sufficient to establish this very interesting point. We are gradually collecting data which indicate the conditions most favorable for stroma production and these experiments will be presented at another time.

The next hemoglobin experiment (table 77) is not very convincing as we do not have a suitable control of the bread and milk factors in this dog. Moreover this is the first anemia experiment on this dog and under such circumstances this reaction is often atypical, as has been noted before. We may say that the blood regeneration due to the bread and milk plus hemoglobin might be identical with a reaction on bread and milk alone. We cannot point with certainty to any difference which can be attributed to the hemoglobin. This experiment is unlike the others of this group which give positive evidence that hemoglobin does influence the curve of blood regeneration. Finally, we must conclude that this experiment does not give any evidence against the value of hemoglobin but it also gives no positive support to the other experiments.

Table 78 presents a long experiment in which hemoglobin is given by mouth during one period and by intravenous injection during a subsequent period. We feel that there is good evidence that hemo-

globin did influence favorably the blood regeneration. The anemia level produced by three bleedings was slightly below the average. The first period of sugar feeding plus 10 grams of hemoglobin by mouth shows a notable gain during 3 weeks. The hemoglobin rises 50 per cent and the pigment volume from 433 to 810. There is a correspond-

TABLE 77

Blood regeneration—powdered hemoglobin and bread and milk. Dog 18–124. Terrier mongrel, female, young adult

DATE, 1918	PIGMENT VOLUME = Hb. PER CENT TIMES BLOOD VOLUME	BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	R. B. C. HEMATOCRIT	Hb.	COLOR INDEX	R. B. C. MILLION	W. B. C.	WEIGHT	BLOOD PER KILOGRAM
		cc.	*cc.*	*cc.*	*per cent*	*per cent*				*kgm.*	*cc.*
5/16		515	273	234	45.5					5.80	89
5/26	Diet: Bread and milk										
5/27	488	548	302	241	43.8	89	0.53	8,4	10,4	6.10	90
5/28	Bled 137 cc.										
5/29	Bled 137 cc. No distress										
5/29	Diet: 125 grams bread (ground and dried), 300 cc. milk, 5 grams hemoglobin*										
5/31	207	398	308	87	21.8	52	0.59	4,4	12,8	5.70	70
6/5	370	481	348	129	26.9	77	0.80	4,8	11,2	5.75	84
6/14	376	522	357	160	30.6	72	0.61	5,9	8,6	5.35	98
6/21	421	513	325	183	35.7	82	0.60	6,8	7,8	5.20	99
6/26	342	417	266	145	34.7	82	0.66	6,2	7,2	5.25	79

* Hemoglobin: Defibrinated blood centrifuged, cells washed twice with N/1 salt solution, 2 volumes distilled water added, allowed to lake over night, centrifugalized, stroma removed, hemoglobin dried and powdered.

No previous anemia experiments on this dog.

ing rise in the red cell hematocrit and red cell count. On sugar alone we recall that the gain in these pigment factors is only trifling, perhaps 10 per cent in hemoglobin and corresponding amounts in the other readings. We must hold the hemoglobin responsible at least for a part of this favorable reaction.

TABLE 78

Blood regeneration—sugar and hemoglobin—crackermeal, milk and hemoglobin. Dog 17–157. Coach mongrel, female, young adult

DATE, 1918	PIGMENT VOLUME = Hb. PER CENT TIMES BLOOD VOLUME	BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	R. B. C. HEMATOCRIT	Hb.	COLOR INDEX	R. B. C. MILLION	W. B. C.	WEIGHT	BLOOD PER KILOGRAM	REMARKS
		cc.	*cc.*	*cc.*	*per cent*	*per cent*				*kgm.*	*cc.*	
8/28	1366	1027	518	504	49.0	133	1.00	6.7	14.4	10.10	102	*Slight
8/28	Diet: Crackermeal and milk											
8/29	Bled 257 cc.											
8/30	Bled 257 cc.											
8/31	565	777	560	215	27.6	73				9.65	81	
8/31	Bled 194 cc.											
9/3	433	731	544	179	24.5	59	0.98	3.0	13.8	9.25	79	*Poik. +
9/10	Diet: 75 grams sugar, 25 grams dextrose, 10 grams hemoglobin† by stomach tube											
9/10	646	848	590	249	29.4	76	0.84	4.5	9.4	8.40	100	*Poik. + Vomited
9/16	750	896	593	294	32.8	84	0.82	5.1	6.8	8.00	113	* Poik. +
9/25	810	788	492	293	37.2	102	0.86	5.9	6.6	7.25	109	* Poik. +
9/25	Diet: 100 grams sugar, 30 cc. hemoglobin intravenously‡											
9/30	625	742	463	271	36.5	84	0.70	6.0	6.2	6.95	106	* Poik. +
9/30	Diet: 200 grams crackermeal, 500 cc. milk, and hemoglobin intravenously‡											
10/11	662	752	465	280	37.2	88	0.76	5.8	13.6	7.55	100	
10/16	805	830	502	322	38.8	97	0.73	6.3	12.8	7.55	110	* Slight
10/16	Diet: 200 grams crackermeal, 500 cc. milk											
10/23	722	902	570	322	35.8	80	0.67	6.0	16.0	8.00	112	* Poik. +

* Poikilocytosis of red blood cells.

† Blood centrifugalized, washed once with salt solution; 2 volumes of distilled water added to washed, packed red blood cells. Allowed to stand 24 hours. Centrifugalized and stroma removed. Dried and powdered.

‡ For injection into the vein: With aseptic technique blood is centrifugalized, washed once with salt solution; 20 cc. distilled water added to 10 cc. washed, packed red blood cells. Allowed to stand 4 hours, added 2.5 cc. of 10 per cent salt solution. Centrifugalized and stroma removed. Total amount injected intravenously daily.

Experimental history, see table 66–b.

When the sugar diet is continued but the hemoglobin is given intravenously, we note a fall in hemoglobin and pigment volume but no change in the red cell hematocrit, red cell count and plasma volume. We have no good explanation to fit these observed facts.

TABLE 79

Blood regeneration—hemoglobin injection intravenously. Dog 17–27. Bull mongrel, female, young adult

DATE, 1918	PIGMENT VOLUME = Hb. PER CENT TIMES BLOOD VOLUME	BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	R. B. C. HEMATOCRIT	Hb.	COLOR INDEX	R. B. C. MILLION	W. B. C.	WEIGHT	BLOOD PER KILOGRAM	REMARKS
		cc.	cc.	cc.	per cent	per cent				kgm.	cc.	
8/9	1914	1454	718	727	50.0	133	0.75	8,7	12,0	16.15	90	
8/9	Diet: Bread and milk											
8/12	Bled 364 cc. No distress											
8/13	Bled 364 cc. No distress											
8/15	749	1040	732	297	28.6	72				15.55	67	
8/15	Bled 260 cc.											
8/17	817	1034	718	311	30.1	79	0.95	4,1	24,6	15.45	67	* Poik.++
8/17	Diet: Hemoglobin injection intravenously;† 75 grams sugar, 25 grams dextrose, 200 cc. water daily by stomach tube											
8/23	908	1080	710	370	34.3	84	0.76	5,5	14,8	14.55	74	
8/30	1155	1100	647	442	40.2	105	0.75	7,0	5,6	13.75	80	* Poik.++
9/2	Accidental death											

* Poikilocytosis of red blood cells.

† Hemoglobin: Blood drawn from normal dog with aseptic precautions. Centrifugalized, washed once with salt solution; 20 cc. distilled water added to 10 cc. washed, packed red blood cells. Allowed to stand 4 hours, added 2.5 cc. of 10 per cent salt solution. Centrifugalized and stroma removed. Total amount injected intravenously daily.

Experimental history, see table 15-b; see sugar control, table 15.

The 2 weeks following on a crackermeal and milk diet plus hemoglobin intravenously show a slight gain in pigment substance. Even this slight gain may have some significance when we recall that it

occurred following a long period of limited diet intake. Hemoglobin regeneration under such unfavorable circumstances is very difficult and becomes increasingly difficult as the limited diet periods are extended.

Table 79 gives an experiment which was unfortunately terminated at the end of 2 weeks by an accident. We are able to refer to a control reaction on sugar feeding alone (table 15). This table shows a slight gain in hemoglobin, red cell hematocrit and pigment volume during the first week on the sugar diet. The second week shows no gain. Compare with this reaction the figures in table 79 which show a gain in the first week which may be compared with the control but the second week intead of the stationary level in the control shows a distinct gain in red cells, pigment volume, hemoglobin, etc. We feel that a part of this gain is to be explained by the hemoglobin injections.

Table 80 illustrates another type of experiment in which the hemoglobin injections are given under conditions very unfavorable for blood regeneration. There is a 3-week period of sugar feeding during which time there is zero gain in pigment substance. There is a slight but distinct gain following 5 days of hemoglobin injection and further slight gains on a crackermeal and milk diet with daily hemoglobin injections. Some of the subsequent gains in hemoglobin, red cell hematocrit and red cells may be due in part to the hemoglobin injections which in all probability cannot be at once utilized. The crackermeal and milk alone or with yeast can account for very little blood regeneration when given subsequent to a long period of sugar feeding. The evidence for the favorable influence of hemoglobin injections is not as strong as in some of the other experiments which have the complete control periods.

The last hemoglobin experiment (table 81) is to be compared with table 76. The hemoglobin in this instance is given intraperitoneally so that the absorption might be rapid and the elimination of slight amounts through the urine be obviated. The influence of a different set of phagocytic cells might well be a factor but the gross results are much the same as regards blood regeneration.

Three weeks of sugar feeding plus hemoglobin injection give an amount of blood regeneration which cannot be explained as due to the sugar feeding. We note a rise of hemoglobin from 72 to 120 per cent and red cell hematocrit from 27 to 45 per cent. The control sugar diet figures would show only trifling gains. Subsequent weeks on a crackermeal and milk diet do not show much gain except in the red count. The final period of mixed diet as usual brings the dog back to a high normal figure.

TABLE 80

Blood regeneration—hemoglobin intravenously. Dog 16-160. Bull mongrel, female, young adult

DATE, 1918	PIGMENT VOLUME = Hb. PER CENT TIMES BLOOD VOLUME	BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	R. B. C. HEMATOCRIT	Hb.	COLOR INDEX	R. B. C. MILLION	W. B. C.	WEIGHT	BLOOD PER KILOGRAM	REMARKS
		cc.	*cc.*	*cc.*	*per cent*	*per cent*				*kgm.*	*cc.*	
8/28	1835	1103	456	654	58.2	166	1.10	7,6	9,6	10.15	109	
8/28	Diet: Bread and milk											
8/29	Bled 276 cc.											
8/30	Bled 276 cc.											
9/1	648	771	524	243	31.5	84				9.55	81	*
9/1	Bled 193 cc.											
9/3	448	696	513	177	25.5	64	0.80	4,0	15,4	9.50	73	* Poik.++
9/3–9/26	Diet: 75 grams cane sugar, 25 grams dextrose, 200 cc. water by stomach tube (table 18)											
9/25	429	664	474	182	27.4	64	0.61	5,2	9,6	7.35	90	* Poik.++
9/26	Diet: Hemoglobin intravenously,‡ 30 cc., and 100 grams sugar by stomach tube											
9/30	437	618	421	194	31.4	71	0.68	5,2	12,4	7.15	86	* Poik.++†
9/30	Diet: Hemoglobin intravenously,‡ 30 cc., and 200 grams crackermeal, 500 cc. milk											
10/11	506	712	477	227	31.9	71	0.58	6,1	11,0	7.75	92	* Poik.++
10/16	586	740	495	237	32.0	79	0.65	6,1	5,0	7.80	95	* Poik.++†
10/16	Diet: Hemoglobin injection discontinued; 200 grams crackermeal, 500 cc. milk											
10/23	670	826	536	281	34.0	81	0.65	6,2	8,4	8.05	103	* Poik.++†
10/23	Diet: 1 gram dried brewer's yeast, 200 grams crackermeal, 500 cc. milk.											
10/30	590	808	536	264	32.7	73	0.47	7,7	15,2	8.30	97	* Poik.++†
11/6	720	868	547	317	36.5	83	0.66	6,3	8,6	8.20	106	* Poik.+
11/12	784	883	532	342	38.7	89	0.50	8,9	7,2	8.25	107	* Poik.+
11/13	Diet: Mixed diet											
11/22	726	853	503	341	40.0	85	0.42	10,2	13,6	8.80	97	* Poik.+
11/29	1122	1020	547	463	45.4	110	0.69	8,0	19,0	9.25	105	Excellent condition

* Poikilocytosis of red blood cells.

† Shadow cells.

‡ Hemoglobin: Blood taken with aseptic precautions, centrifugalized, washed once with salt solution; 20 cc. of distilled water added to 10 cc. washed, packed red blood cells. Allowed to stand 4 hours, added 2.5 cc. of 10 per cent salt solution. Centrifugalized and stroma removed. Total amount injected intravenously daily.

Experimental history, see table 18-b.

TABLE 81

Blood regeneration—hemoglobin intraperitoneally. Dog 17–28. Bull mongrel, female, young adult

DATE, 1918	PIGMENT VOLUME = Hb. PER CENT TIMES BLOOD VOLUME	BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	R. B. C. HEMATOCRIT	Hb.	COLOR INDEX	R. B. C. MILLION	W. B. C.	WEIGHT	BLOOD PER KILOGRAM	REMARKS
		cc.	cc.	cc.	per cent	per cent				kgm	cc.	
8/9	2417	1580	680	887	56.1	153	0.84	9,1	14,2	17.00	93	
8/9	Diet: Bread and milk											
8/12	Bled 395 cc. No distress											
8/13	Bled 395 cc. Diet: Crackermeal and milk											
8/15	1015	1142	750	387	33.8	89				16.30	71	
8/15	Bled 286 cc.											
8/17	756	1050	758	281	26.8	72	0.97	3,7	23,6	15.90	66	* Poik.+
8/19	Hemoglobin† (intraperitoneal injection). Diet: 75 grams sugar, 25 grams dextrose, water											
8/23	794	1030	708	310	30.1	77	0.69	5,6	21,4	15.40	67	* Poik.
8/30	1133	1193	685	430	36.0	95	0.76	6,2	12,6	14.55	82	* Poik.+
9/4	Hemoglobin† (intraperitoneal injection). Diet: 100 grams sugar in 500 cc. water											
9/6	1670	1385	755	608	45.0	120	0.83	7,2	11,0	13.40	103	* Poik.++
9/6	Hemoglobin discontinued. Diet: 200 grams crackermeal, 500 cc. milk kaolin											
9/13	1550	1450	876	566	39.2	107	0.68	7,9	9,8	14.00	103	
9/19	1433	1310	752	545	41.6	109	0.71	7,7	12,2	13.90	94	* Poik.
9/27	1253	1130	658	468	41.4	111	0.71	7,8	10,6	14.30	79	* Poik.
10/9	1635	1363	668	682	50.5	120	0.77	7,8	12,2	14.20	96	
10/18	1836	1386	680	694	50.0	132	0.66	10,1	9,0	14.45	96	
10/25	1550	1352	704	636	47.0	115	0.64	9,0	10,8	14.60	93	
10/25	Diet: 200 grams crackermeal, 500 cc. milk, 1 gram dried, powdered brewer's yeast, kaolin											
10/31	1415	1400	750	638	45.4	101	0.65	7,8	11,8	15.00	93	
10/31	Diet: Mixed diet											
11/7	1975	1555	710	829	53.3	127	0.74	8,6	10,8	15.10	103	
11/13	2000	1600	743	840	52.5	125	0.63	9,9	20,2	15.05	106	

* Poikilocytosis of red blood cells.

† Hemoglobin: 10 cc. sterile, washed, packed red blood cells and 20 cc. distilled water. Centrifugalized and stroma removed. Intraperitoneal injection.

Experimental history, see table 6–b; see table 76 for hemoglobin feeding.

SUMMARY

Blaud's pills are inert when added to various diets which do or do not favor rapid blood regeneration. We may not assume without positive proof that inorganic iron is of value in the treatment of secondary anemia.

Splenectomy may or may not modify this blood regeneration reaction. Limited diets following anemia periods associated with splenectomy may be the cause of fluctuations in the normal expected curve of blood regeneration.

Hemoglobin (by mouth, intravenously or intraperitoneally) exerts a distinctly favorable influence upon subsequent blood regeneration.

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PHYSIOLOGIC CHANGES PRODUCED BY VARIATIONS IN LUNG DISTENTION

III. Impairment of the Coronary Circulation of the Right Ventricle

RALPH HOPKINS and FELIX P. CHILLINGWORTH

From the Physiological Laboratory, Tulane University of Louisiana, New Orleans

Received for publication June 1, 1920

In a previous communication a method was described by which the maximum blood pressure obtainable in the pulmonary arteries could be measured by the increase in intrapulmonic air pressure sufficient to prevent the passage of blood through the capillaries of the lungs (1). The mechanical blocking of the pulmonary circulation produced under these conditions was found to cause a maximum pulmonary arterial pressure of 85 mm. of mercury and a coincident complete disappearance of carotid pressure. In the present communication we record the results of some experiments to determine the critical point during progressive increase of intrapulmonic air pressure at which circulation of blood through the coronary arteries of the right ventricle becomes impossible because of the maintenance of a level of blood pressure in the pulmonary arterial and right cardiac systems equal to or higher than that of the general systemic circulation. Distention of the lungs to or beyond this critical point is almost immediately fatal and over-distention even when not attaining this critical degree also interferes with adequate oxygenation and nutrition of the heart. The necessity for a balance in favor of the diastolic systemic pressure over the diastolic right ventricular pressure has a bearing on artificial respiration effected by increase in the intrapulmonic air pressure. Loss of this balance through over-distention of the lungs may defeat the purpose for which artificial respiration is given by causing asphyxiation of the heart muscle even while the lungs are being over-ventilated. This observation is especially pertinent in the conditions in which, as is usually the case when artificial respiration is resorted to, the systemic arterial pressure is low and but little further fall is sufficient to reduce

it below the level of pressure in the pulmonary arteries because the latter rises during great distention of the lungs while the systemic arterial pressure falls.

An endeavor is also made to show that even with the lungs distended in lesser degree than the critical point referred to, death may result owing to impairment of the coronary circulation of the right ventricle.

METHOD

The method employed to increase the pressure of the intrapulmonic air has been described in a previous communication (2). The use of the plethysmograph referred to facilitates the attainment of the degrees of pressure desired and obviates difficulties that would arise from forced expiratory efforts of the animals if the usual methods of intratracheal insufflation were employed. By placing the entire animal within the plethysmograph and connecting his trachea to the outside air, diminution of the pressure within the plethysmograph produces the same effects on the animal's relation to the air within his lungs as would result from an equal increase in the intrapulmonic air pressure while the exterior of the animal remained under atmopheric pressure. The decrease, therefore, in plethysmographic pressures is an exact measure of the actual increase of intrapulmonic air pressures and in referring to the effects of these pressures the terms are regarded as interchangeable.

A correction must be made in measuring blood pressures which are recorded during diminished plethysmographic pressures. To the blood pressures recorded in millimeters of mercury should be added the difference, in millimeters of mercury, existing between the atmospheric pressure and the pressure within the plethysmograph. The necessity for this correction is made obvious by the fact that with diminished plethysmographic pressures the carotid pressure may fall considerably below its zero line.

For recording blood pressures and the pressure within the plethysmograph mercury manometers were used and the readings in millimeters of mercury on the unreduced tracings have been multiplied by 2 when used in the subsequent paragraphs.

To record the pulmonary arterial pressure a branch of the left pulmonary artery was used and artificial respiration by intratracheal insufflation was performed after opening the chest and continued until the animal was placed in the plethysmograph. In the intervals

between experiments, artificial respiration was maintained by rhythmical changes in the plethysmographic pressure.

Recovery after distention of the lungs is rapid and many experiments may be performed on one animal but the time factor as regards the duration of lung distention is of importance. In obtaining the results recorded in subsequent paragraphs no experiment exceeded one minute in duration.

RESULTS OF EXPERIMENTS

Four typical tracings are reproduced taken from a series of 25 experiments performed on 10 dogs, and a curve is plotted to show the effect of lung distention on the carotid and pulmonary arterial pressures. The tracings and the plot are first described and the results on the coronary circulation are subsequently discussed.

Figure 1. Synchronous records of the carotid and pulmonary arterial pressures are reproduced. The pressure within the plethysmograph is also shown. Air was exhausted from the plethysmograph during a period of one minute effecting a gradual fall in the intraplethysmographic pressure to 83 mm. of mercury below the atmospheric pressure. On the unreduced tracing from which this figure is reproduced the maximum fall in carotid pressure was equal to 212 mm. of mercury (actually 46 mm. below the carotid zero line), while the fall in pulmonary pressure was equal to 54 mm. of mercury (9 mm. below its zero line). The carotid pressure shows a corrected fall of 129 mm. (212–83), while the pulmonary pressure when corrected shows actually a rise of 29 mm. of mercury. When corrected, the minimum carotid and maximum pulmonary pressures are respectively 41 (preëxperimental pressure level 170 minus corrected fall 129) and 79 (preëxperimental level 50 plus rise resulting from correction 29). Respiratory waves, following a period of apnoea which was of 18 seconds' duration, are marked in both arterial curves but show only slightly in the plethysmographic record, since they were the result of spontaneous but ineffectual efforts on the part of the animal. It will be noted that during the last 14 seconds of the experiment the intraplethysmographic pressure rose slightly and that with this increase in pressure there occurred a rise in carotid while the pulmonary pressure continued to fall. It will also be noted that as the plethysmographic pressure is progressively lowered there is a gradual disappearance of pulse pressure in both the arterial curves. With sudden return to atmospheric pressure within the plethysmograph there is an almost

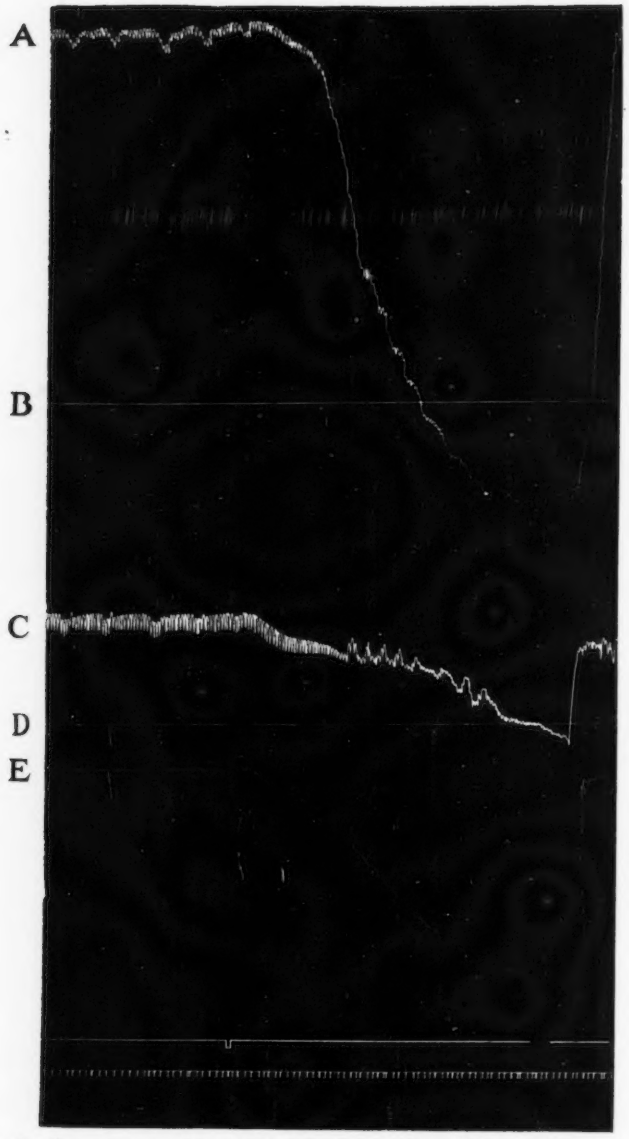

Fig. 1. Experiment of March 20, 1916. Dog 6 kilo. Ether. Time in seconds. *A*, Carotid blood pressure recorded by mercury manometer; *B*, Zero level for carotid blood pressure; *C*, Left pulmonary blood pressure recorded by mercury manometer; *D*, Zero level for pulmonary pressure; *E*, Plethysmographic pressure recorded by mercury manometer which is 68 mm. above the time record.

synchronous recovery of both arterial pressures to their preëxperimental pressure levels.

Figure 2. Records of carotid and pulmonary arterial pressures are reproduced from three parts of a typical tracing showing the effects

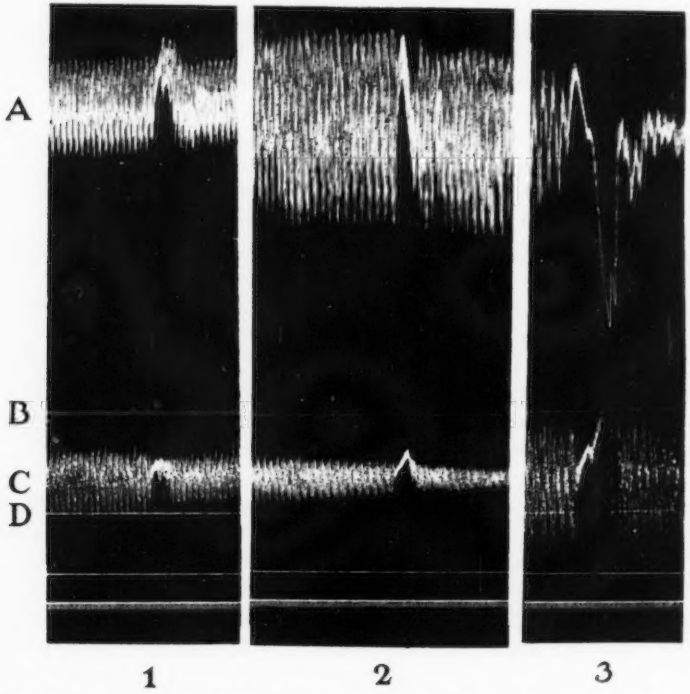

Fig. 2. Experiment of January 5, 1918. Dog 8.75 kilo. Ether. Time in seconds. *A*, Carotid blood pressure recorded by mercury manometer; *B*, Zero level for carotid blood pressure; *C*, Left pulmonary blood pressure recorded by mercury manometer; *D*, Zero level for pulmonary pressure. This level is 20 mm. above the time record. *1*, *2* and *3* show the progressive effects of increasing lung distention.

of distention of the lungs in varying degree. The experimental procedure was varied in the experiment on this animal and the plethysmograph was not used. The lungs were inflated through the trachea which was connected to a reservoir containing air under pressure. The degree of pressure employed to distend the lungs is not recorded but

can be estimated from the effects produced upon the arterial pressures. Small caliber mercury manometers were used for recording blood pressures.

In the first of these tracings the lung distention is quite moderate and is accompanied by a slight rise in both arterial pressures. The systolic pulmonary pressure shows no rise but the mean blood pressure is higher due to maintenance of a higher diastolic level. Measurements of the unreduced tracing show a preëxperimental mean pulmonary pressure of 14 mm. of mercury which during inflation rises to 20.

In the second of this series the distention of the lungs is slightly greater and the mean pulmonary pressure rises from a preëxperimental level of 16 to 28 mm. of mercury. In this instance the systolic as well as the diastolic pressure rises during distention of the lungs, but the rise in the systolic is 8 mm. less than the rise in diastolic pressure.

In the third tracing distention of the lungs is in excess of that in either of the previous experiments and a corresponding increase is observed in the pulmonary arterial pressure which attains a maximum mean pressure of 43 mm. (29 mm. above the preëxperimental level of 14). Again it is noticeable that the rise in the diastolic exceeds that in the systolic pressure. A comparison of the mean pulmonary to the mean carotid pressures shows that, at the point of greatest lung distention, the pulmonary pressure exceeds that in the carotid artery by 6 mm. and also that the pulmonary diastolic pressure is slightly in excess of that of the carotid. The initial carotid pressure in this animal is 40 mm. lower than that plotted in figure 3, and as a result of this lower preëxperimental level a comparatively small fall in carotid pressure is sufficient to make the carotid equal to the pulmonary arterial pressure.

Figure 3. A curve is plotted from a typical tracing showing simultaneous carotid and pulmonary arterial pressures in millimeters of mercury during progressive increase of intrapulmonic air pressure. The broken line represents the former and the unbroken line the latter. The figures used have been corrected as explained in a previous paragraph. The ordinates indicate blood pressures and the abscissae the pressures in millimeters of mercury employed to distend the lungs.

The carotid pressure, while distention is progressing, shows an initial rise of 6 mm. of mercury attained when the excess of air pressure within the lungs is equal to 10 mm. of mercury. With increasing distention of the lungs the carotid pressure falls progressively, the maximum fall being attained with an increase in the intrapulmonic air

pressure equal to 80 mm. of mercury, which was the greatest pressure used. With the exception of the small initial rise accompanying slight distention of the lungs, the fall in carotid pressure below the preëxperimental level of 170 mm. progresses uniformly with increasing lung distention until the low level of 36 mm. is reached.

The pulmonary arterial pressure, on the contrary, is not noticeably affected by slight distention of the lungs, and with increasing distention rises continuously until its maximum is attained, which occurs when the increase in the intrapulmonic air pressure is equal to 60 mm. of mercury. Beyond this point the pulmonary pressure commences to fall. The maximum increase in the pulmonary pressure is 40 mm. above the preëxperimental level of 50 mm. and the relatively high level of 90 mm. is attained.

It will be noted that the pulmonary pressure rising from a low level becomes equal to the carotid pressure falling from a high level at a point where the pressure in the two systems is equal to 85 mm. The increase in intrapulmonic air pressure sufficient to effect this equality is 50 mm. of mercury. With increase in air pressure in excess of 50 the pulmonary pressure continuing to rise attains a level 46 mm. above that of the falling carotid pressure.

An interesting observation is that both the fall in carotid and the rise in pulmonary pressures are, within a wide range of lung distention, approximately linear functions of the increasing intrapulmonic air pressure.

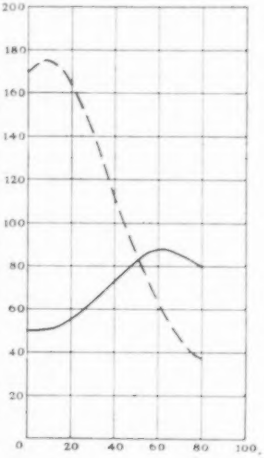

Fig. 3. Plotted curves showing corrected carotid (broken line) and pulmonary (unbroken line) arterial pressures during progressive decrease of intraplethysmographic pressure. Ordinates indicate blood pressures. Abscissae indicate decrease of plethysmographic pressures in millimeters of mercury.

DISCUSSION OF RESULTS

While the pulmonary arterial pressure cannot under usual living conditions be taken as an index of the right ventricular diastolic pressure, yet, under the condition of completely blocked pulmonary capillaries the right ventricular pressure must rise *pari passu* with the rise in

pressure in the pulmonary arteries and this rise must be sustained in the ventricle even during its diastole. Blood under high pressure is trapped between the blocked capillaries at one end of the system and the tricuspid valve at the other. When the pressure in the pulmonary artery equals the maximum which the right ventricle is capable of establishing, subsequent contractions are unable to discharge blood, and the ventricle also remains constantly full of blood under a pressure equal to that in the pulmonary arteries. Even during diastole no relief from this excessive pressure is obtained since the ventricle cannot, even though relaxed, increase its capacity for holding blood. It is even quite possible that under these conditions the pulmonary semilunar valves fail to close, since the normal tendency of the blood to regurgitate is absent when the ventricle is full of blood at the end of its systole. In evidence of the diminished output of blood from the right side of the heart when the pulmonary capillaries are mechanically blocked by distention of the lungs, a tracing (fig. 1) is shown in which the pulse is obliterated in the record from the pulmonary artery while the mean pulmonary pressure rises. Obliteration of the pulse waves occurs in the carotid as well as in the pulmonary tracing but in one case it is due to insufficient and in the other to excessive pressure.

When the pulmonary capillaries are completely blocked the pulmonary arterial pressure may then be taken as a direct measure of the diastolic right ventricular pressure and it may be assumed that with an incomplete blocking of the capillaries there exists a rise in the diastolic right ventricular pressure corresponding in degree to that maintained in the pulmonary arteries. Two of the tracings shown in figure 2 are evidence of the fact that with moderate distention of the lungs the rise in mean pulmonary arterial pressure is due to a rise in diastolic rather than in systolic pressure. In the third tracing of this series it will be noted that as the pulmonary blood pressure rises the output from the ventricle diminishes. That the diminution in ventricular discharge is not due to a diminished supply of blood to the auricle and ventricle was evidenced by many post-mortem examinations which revealed not only a dilated right ventricle but also an engorgement limited to the right auricle and adjacent veins in those animals that were killed by excessive increase of intrapulmonic pressure. It will be noted in figure 3 that after attaining its maximum the pulmonary arterial pressure commences to fall. This failure is attributable to cardiac failure secondary to impairment of the coronary circulation. Marked fall in carotid pressure necessarily affects the coronary circu-

lation of the entire heart but when the right ventricle is distended during its diastole with blood under high pressure, the impairment of circulation through the cardiac muscles is far greater in the right ventricle than in other parts of the heart.

Taking the pulmonary arterial pressure as an index of safety during distention of the lungs, reference to figure 3 shows that with 50 mm. increase in the pressure of the intrapulmonic air the carotid and pulmonary arterial pressures become equal and that with increasing air pressure the pulmonary pressure becomes higher than that in the carotid artery. The limit of safety, therefore, in dogs lies below this degree of pressure and death has been found not infrequently to follow air pressures of 30 and even less.

There is no reason to believe that the pulmonary capillaries of man are more resistant to occlusion than are those of the dog or that there exists much difference in their relative blood pressures. Difference in the elastic factor of thoracic expansion is not important since increase in the intrapulmonic air pressure acts almost directly on the lung capillaries independently of lung expansion. The observations, therefore, made on dogs require little or no modification to become pertinent to artificial respiration performed on man by any of the methods that increase intrapulmonic air pressure. When the lungs are rhythmically distended an important factor of safety is the shortening of the period of full inflation to allow the blood pressures to return to normal.

CONCLUSIONS

1. When in dogs the lungs are artificially distended by an increase in the intrapulmonic air pressure:

a. The pulmonary arterial pressure becomes equal to that of the general systemic arterial pressure when the excess of intrapulmonic pressure is equal to 50 mm. of mercury.

b. With further increase in intrapulmonic pressure the pulmonary arterial pressure may exceed that of the general system by as much as 46 mm. of mercury.

c. Under both of the above conditions the circulation of blood through the coronary arteries of the right heart is unfavorably influenced.

d. With the rise in pulmonary arterial pressure there occurs a rise in the diastolic right ventricular pressure. When the latter becomes equal to or greater than the systemic diastolic pressure, circulation of

blood through the coronary vessels of the right ventricle becomes impossible.

2. Estimates of the increase in intrapulmonic air pressure sufficient to influence unfavorably the coronary circulation of dogs are applicable also to man and should be considered in connection with methods of artificial respiration.

We are indebted to Prof. W. E. Garrey for helpful suggestions and criticisms.

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VAGUS AND SPLANCHNIC INFLUENCE ON THE GASTRIC HUNGER MOVEMENTS OF THE FROG. COMPARATIVE STUDIES III[1]

T. L. PATTERSON

From the Hull Physiological Laboratory, The University of Chicago, and the Physiological Laboratory, Queen's University

Received for publication June 4, 1920

INTRODUCTION

The character of the continuous motor activity of the empty and filled stomach in the frog has been reported (1). In the present paper an attempt is made to determine more specifically the influence of the vagi and splanchnic nerves on the behavior of the gastric hunger movements and the gastric tonus of the empty stomach.

Much has been written concerning the excitatory and inhibitory influences of these extrinsic nerves upon the gastric motility and a rather thorough review of the literature covering this phase of the question has appeared in a previous paper of this series (1). In addition, the distribution and function of the nerves innervating the visceral and vascular systems in crocodiles and alligators was worked out by Gaskell (2). He found that stimulation of the peripheral end of either vagus above or below the ganglion trunci vagi invariably led to a contraction of the stomach musculature. After section of the cervical vagus above the ganglion with subsequent degeneration of its fibers stimulation then above the ganglion almost invariably produced no effect whatever on the esophagus and stomach, while stimulation below the ganglion almost invariably caused marked peristaltic contraction. Gaskell came to the conclusion that the fibers which innervate the thoracic portion of the esophagus and the stomach and all probability the intestines degenerate only in that portion which is above the ganglion but not in that portion below the ganglion and that the nerves for the

[1] A preliminary report of this work was made before the 1917 meeting of the American Physiological Society at Minneapolis, a brief abstract of which was published in the Proceedings of that Society.

upper part of the esophagus and the inhibitory fibers of the heart have no connection with the nerve cells of the ganglion trunci vagi, while the motor nerves for the rest of the esophagus and the upper portion of the remainder of the alimentary canal are in connection with the cells of that ganglion—a connection by which the motor fibers proceeding peripherally from the ganglion are prevented from degeneration but not the motor fibers which pass to the ganglion.

Furthermore, the recent observations by Crohn and Wilensky (3) on gastric behavior by the balloon method have shown that in atony of the stomach the hunger contractions disappear and in advanced cases the tonal waves also, while in purely secretory or other functional disturbances both kinds of waves persist.

EXPERIMENTAL PROCEDURE

The studies in this series of experiments were made upon the large bullfrog (Rana catesbiana). All the animals were provided with an artificial opening into the posterior part of the mouth or stomostomy and the movements of the empty stomach were recorded by the balloon method as described in a previous paper (4). A series of normal contractions of the empty stomach was obtained from each animal which extended over a period of several days and then each of these animals was operated on a second time. In this second operation, either both vagi or both splanchnic nerves, or both the vagi and the splanchnic nerves together were sectioned, followed after recovery in each case by a series of tracings from the empty stomach. The animals were anesthetized. Aseptic precautions as far as possible were at first followed but later this was found to be unnecessary as no infection developed in any of the animals when such procedure was not followed. The vagi were sectioned in the region of the neck. Two oblique incisions were made through the skin on either side of the median line, ventral, about 1 cm. distant and close to the anterior tips of the shoulders as represented by a line drawn from this point laterally 1 cm. to $1\frac{1}{4}$ cm. in length to a point slightly posterior and just internal to the articulation of the superior and inferior maxillary bones on either side. These two incisions exposed the cervical fascia on either side at its attachment along the anterior scapulo-clavicular borders. Here there are few blood vessels and if the fascia is carefully separated no hemorrhage results. As soon as this region is passed the fascial separation becomes very easy until the thin sheet of prevertebral fascia is reached

which is about on a line of the transverse processes passing obliquely downward and inward from the base of the skull and extending into the thorax. This latter sheet of fascial membrane is now pierced which exposes the levator anguli scapulae muscle, over the anterior border of which courses the vagus nerve and the internal jugular (Vena jugularis) and musculo-cutaneous (Vena musculo-cutanea) veins. The incision is held open by the spring of a small pair of forceps (preferably curved points) and then by means of a small pair of mouse-toothed forceps the nerve is carefully separated from the adjoining veins to which it is bound by connective tissue. This is best accomplished by freeing the nerve either between the two veins mentioned or just lateral to the internal jugular vein at the anterior border of the levator anguli scapulae where it crosses and sectioning the nerve just below the origin of the recurrent laryngeal branch. Section of the nerves at this point destroys not only the gastric branches to the stomach but also the pulmonary and cardiac branches destined for the lungs and heart. Attempts were made at first to section only the gastric branches but as these branches were so small and so deeply embedded in the tissues it was found to be practically impossible with recovery of the animals. In fact, the technic as used required several months' experience before it became perfected and only then did it become an efficient procedure which, if properly handled, may be called a bloodless method. Both vagi were always sectioned at one operation and the skin incisions were closed with five sutures.

The splanchnic nerves were sectioned in the region of the coeliac plexus after laparotomy. An incision was made through the skin, the rectus abdominis muscle and the aponeuroses of the external and internal oblique muscles $2\frac{1}{2}$ to 3 cm. in length extending from the lower extremity of the sternum (xiphisternum) caudalward and about $\frac{1}{2}$ cm. to the left of the linea alba, in order to avoid the anterior abdominal vein (Vena abdominalis) which courses forwards along the mid-line of the ventral body wall until opposite the liver. The stomach is withdrawn through this opening and a larger pair of nerves, one on either side, is found coursing along with the right and left systemic arches. These are the third spinal nerves carrying fibers for the stomach, but according to Steinach and Wiener (5), Dixon (6) and others, the stomach also receives fibers from the fourth and fifth spinal nerves and Waters (7) in addition includes the sixth.

Fibers from these nerves arising from both sides of the body unite to form the coeliac plexus situated on the coeliaco-mesenteric artery

(Ateria intestinalis communis) a few millimeters from its origin from the left systemic arch. From this plexus arise the nerves destined for the stomach, pancreas and duodenum. The branches for the stomach are inbedded in the mesenteric membrane and follow closely the course of the arterial supply of this organ. They may be best seen by raising the stomach and allowing the light to illuminate the mesentery when they may be picked up with mouse-toothed forceps. If they are cut close to the plexus there are usually not more than two branches. Experience has shown that it is advisable to introduce into the stomach 5 or 6 cc. of water previous to the operation as it tends to fill and round out the stomach, thus making it easier to locate the plexus and the nerves. The technic for this operation like that for double vagotomy in the frog is very delicate, but with sufficient patience and experience it may be developed to such a point as to be conducted without hemorrhage, and like the former may be considered a bloodless method. The splanchnetomized stomach is pushed back into place, the muscular incision is closed with nine to ten sutures and the skin incision with the same number. The animals after double vagotomy or splanchnetomy are usually sufficiently recovered on the third day following the operation to be used for experimental tests with fairly marked gastric activity, while after a double operation consisting of the two above they are usually not ready for use until the fourth day following the operation. In the decerebration experiments the balloon was not removed from the stomach and the gastric contractions started again after a short period of inhibition. All the tracings were recorded on a slowly moving drum making a revolution in fifty to sixty minutes.

THE INFLUENCE ON THE GASTRIC HUNGER MOVEMENTS OF PARTIAL AND COMPLETE ISOLATION OF THE STOMACH FROM THE CENTRAL NERVOUS SYSTEM

A complete knowledge of the mechanism of the gastric movements is still uncertain. The gastric activity is regulated not only by the vagi and the splanchnic nerves of the sympathetic system, but also by the automatically acting plexi of Auerbach and Meissner. The most direct and desirable method of attack on this problem is the section of the extrinsic nerves to the stomach, although this operation abolishes not only all direct influences from the brain of a motor or inhibitory type, but also the central reflexes (motor or inhibitory) that may be called into action through the sensory nerves in the stomach.

The influence of these nerves on the activity of the stomach has been studied by a number of investigators, prominent among whom have been Cannon (8) and Carlson (9). Cannon's observations on the gastric movements of digestion in cats have shown that section of the vagi leads to a temporary loss of tonus and a slowing and weakening of the peristalsis, which in respect to rate is practically restored in a few days. He infers as does Kelling (10) that their function is solely to make the gastric muscles exert a tension (tonic state) and the result of this condition is peristalsis. Furthermore, section of the splanchnic nerves does not affect the movements of digestion, while the combined vagi and splanchnic section leaves the digestive movements of the stomach practically normal even shortly after the operation.

Carlson, on the other hand, has shown that section of the vagi in dogs leaves the empty stomach on the whole permanently hypotonic, at least for a period up to three months after the operation. Section of the splanchnic nerves increases the gastric tonus and augments the gastric hunger contractions, while the section of both the vagi and the splanchnics leads to a permanent hypotonus of the stomach, except under conditions of prolonged fasting. These discrepancies between the results of the two investigators are probably accounted for, in that the tonus of the vagus plays a greater rôle in the movements of the empty than in the movements of the filled stomach, or else the nerves vary in different species of animals.

1. The effect of complete section of the splanchnic nerves. Complete section of the splanchnic nerves on both sides in the region of the coeliac plexus was made on twelve frogs and after recovery from the operation records of the movements of the empty stomach were continued from two to three weeks and compared with those from the normal stomach of the same animal.

When a comparative study of the records of these animals is made as a whole it is evident that the complete section of the splanchnic nerves with the vagi intact in frogs increases markedly the gastric tonus and augments the movements of the empty stomach (fig. 1, *A* and *B*). The recorded contractions are small, rapid and irregular in form, and represent virtually an incomplete or hunger tetanus of the stomach. In other words, the stomach on the whole becomes strongly hypertonic and more active through the destruction of the inhibitory fibers via splanchnic nerves to the stomach, which permits the motor fibers of the vagi to exert their full influence on the gastric motor mechanism, thus leading to a high degree of gastric tonus much above the normal.

This particular state of excessive tonus is evidenced not only by the balloon in the stomach cavity but more especially by the marked contraction of the esophagus and stomach as is exhibited many times by the extreme difficulty to introduce the balloon through the esophagus into the stomach. This hypertonic condition of the frog's stomach which always appears after complete section of the splanchnic nerves is evidently more marked than Carlson (9) found it to be in dogs after splanchnic section. Furthermore, this condition as it exists in frogs after this type of nerve section corresponds apparently to certain clinical

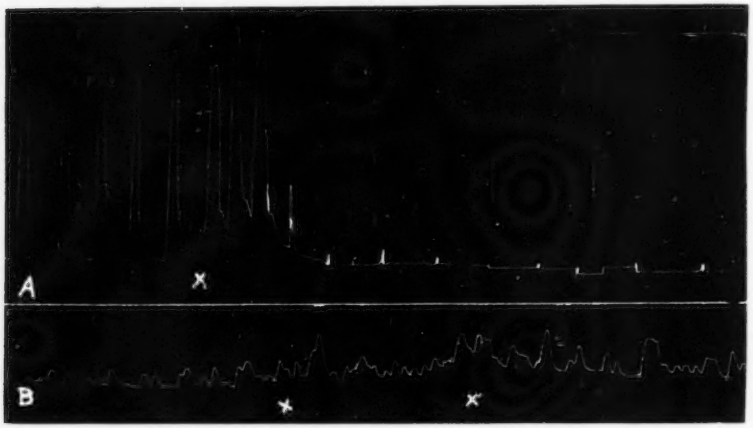

Fig. 1. Records from the empty stomach of the frog. *A*, normal frog after six days' fast; *B*, the same animal ten days after section of both splanchnic nerves and twenty days' fast. At *x*, introduction of 5 cc. of 0.5 per cent solution of hydrochloric acid directly into the stomach. Showing incomplete tetanus and only slight inhibition of the hunger contractions by acid in the stomach after section of the splanchnic nerves. x' = termination of the acid injection.

conditions as reported by Eppinger and Hess (11) under the term of vagotonia. According to these observers the antagonistic influences between the gastric branches of the vagi and the spanchnic nerves play a very important rôle in not only moderating the physiological impulses which might reach a very marked intensity, but in addition they prevent acute transitions from rest to excitation or vice versa. This means that if it were not for the above under certain conditions small stimuli might cause large reactions either physiological or pathological. Now,

if we assume that somewhere in the central nervous system there exists a common center which controls the antagonistic actions of these two systems, as suggested by these investigators, and that the irritability of this center increases and decreases from time to time it is easy to understand how very weak and even transitory stimuli might act upon such a center when in a state of increased irritability to produce the gastric hypertonus through the fibers of the vagi. This however is not proven, but we do know that in the condition of vagotonia there is a functional increase of tone via vagi to the stomach and this increase of function doubtless permits the stimuli to act more readily than if the reversed condition existed. Furthermore, the observations of Crohn and Wilensky (3) have shown that the hunger contractions in well-marked cases of vagotonia exhibit an extreme degree of variability, the contractions following one another in rapid succession and without pause for comparatively long periods of time. The clinical findings of these observers are apparently in accord with the results on frogs after splanchnic section.

The inhibition of the movements of the empty stomach of the splanchnetomized animal when acid is introduced into the stomach cavity is much less complete than in frogs with all the extrinsic gastric nerves intact (fig. 1, *A* and *B*). In fact, the contractions do not cease at all and the only effect produced is a very slight decrease in the height of the contractions during the introduction of the acid followed by a few contractions of a slightly longer duration and usually a slight increase in the gastric tonus. This diminution of the inhibition following stimulation of the gastric mucosa by acids after complete section of the splanchnic nerves is confirmatory with the findings of Carlson (12) on dogs.

2. *The effect of section of both vagi nerves.* Section of both vago-sympathetic nerves in the neck was made on eleven frogs and after recovery from the operation records of the movements of the empty stomach were continued from two to three weeks and compared with those from the normal stomach of the same animal.

When all the records are compared from these animals the results are confirmatory in showing that the contractions of the empty stomach are only slightly changed in rate and regularity. The contractions when viewed as a whole resemble those from the normal stomach with the exception that they usually appear to be of a slightly slower rate, weaker and more irregular (fig. 2, *A* and *B*). However, there is a tendency for the contractions to increase in strength or rather amplitude up to the amplitude of the normal contractions and some of the individual

contractions may even exceed the normal. This is evidently produced through a lowered tone in the gastric motor mechanism, whereby the contractions start rather suddenly and without any marked preliminary increase in tonus and because of this condition the air is more completely forced out of the balloon, thus resulting in the greater contraction. In

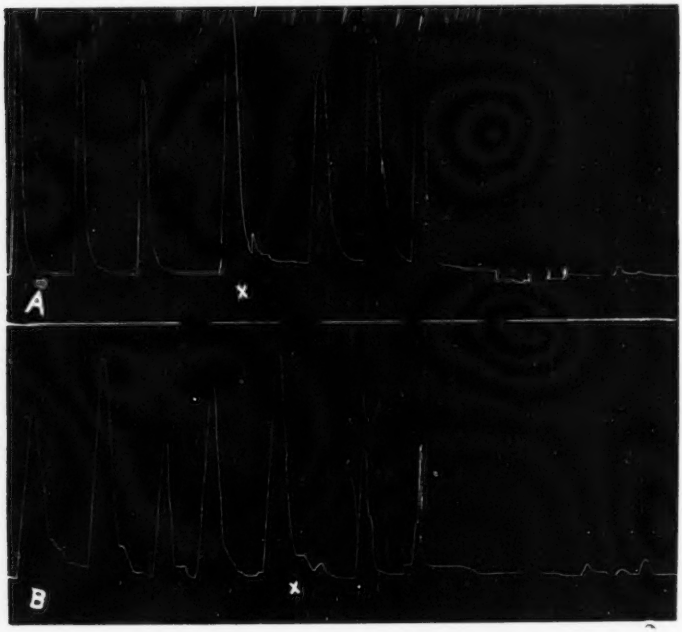

Fig. 2. Records from the empty stomach of the frog. *A*, normal frog after three days' fast; *B*, the same animal nine days after section of both vagi and sixteen days' fast. At *x* introduction of 5 cc. of 0.5 per cent solution of hydrochloric acid directly into the stomach. Showing slightly more complete inhibition of the hunger contractions by acid in the stomach after section of the vagi nerves.

the empty stomach of the normal animal as determined by the balloon method, there are practically no tonus changes, or at least they are so slight in degree as to be almost a negligible factor. In working with these animals, extending over a period of five years, I have never observed in the normal animal an increase in gastric tone exceeding a cen-

timeter as determined by the manometric pressure. This maximal increase I have observed not more than three or four times in my work during this time and is therefore rare. When tonus changes are observed they usually do not exceed a quarter of a centimeter (2 to 3 mm.), but the more common thing in the frog is to have the tonus remain constant hour after hour.

Section of both vagi with the splanchnics intact leads to a sympathetico-tonic condition of the stomach. This means that the stomach on the whole becomes hypotonic through the destruction of the vagal fibers that maintain the gastric tonus, which permits the inhibitory fibers of the splanchnics to exert a greater influence on the gastric motor mechanism, thus leading to a general diminution in the gastric tonus. However, there was a tendency in some of the animals, at least, to show a gradual improvement in the efficiency of the local tonus mechanism as time went on after the operation, which indicates that the hypotonic condition of the stomach may be only temporary in the frog and not permanent as reported by Carlson (9) in dogs, but corresponding to the observations of Cannon (8) in cats for the movements of digestion. This phase of the question will be discussed in a separate paper. Furthermore, the gastric tonus on the whole is much lower than normal as determined not only by the balloon in the gastric cavity, but also by the ease with which the balloon may be introduced through the esophagus into the stomach and inflated.

When acids are introduced directly into the empty stomach of the vagotomized animal gastric inhibition is exhibited similar to that produced in the normal animal with the exception, on the whole, that it appears to be quicker and more marked than in the normal animal (fig. 2, *A* and *B*). This is exactly contradictory to Carlson's results on dogs (9), yet he states that this was what he expected to find, namely—an augmentation of the inhibition through the splanchnics after section of the vagi.

3. The effect of complete section of the vagi and splanchnic nerves. Combined splanchnic and vagi sections were made on ten frogs and after recovery from the operation records of the gastric movements were continued from two to three weeks and compared with those from the normal stomach of the same animal. Both sets of nerves were sectioned at the same operation.

After this complete isolation of the frog's stomach from the central nervous system the movements of the empty stomach are much the same as when the vagi alone are severed. The contractions show a

tendency to approach or even in some cases to exceed the normal, while at times they may even be identical in rate and character with those of the intact stomach, but on the whole they are of a slightly slower rate and more irregular. The stomach passes into a hypotonic condition similar to that after section of the vagi, and therefore the slight changes in the movements of the empty stomach after isolation from the central nervous system must be due primarily to the persistent hypotonus. These results are in general confirmatory with those of Cannon (8) on cats and Carlson (9) on dogs.

The inhibition of the movements of the empty stomach by acid stimulation of the gastric mucosa persists after complete isolation of the stomach from the central nervous system, but the inhibition like that found by Carlson (12) in dogs is diminished in intensity and duration. There is a gradual and slow diminution, both in the rate and amplitude of the hunger contractions but as a rule this does not produce complete inhibition in the frog. The inhibition is therefore primarily a local reflex determined by the local gastric mechanism rather than by the character of the central innervation or the central inhibition. Since the type of gastric activity after complete isolation of the stomach from the central nervous system does exhibit the typical movements of the empty stomach, the primary stimulus to these contractions is not to be sought in the extrinsic nerves. The extrinsic nerves (vagi and splanchnics) must therefore be considered under normal conditions to play the important rôle of modifying or regulating a primary automatic mechanism in the stomach wall.

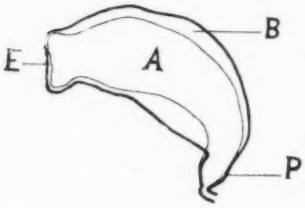

Fig. 3. Splanchnetomized stomach superimposed upon the vagotomized stomach from two frogs of equal size, weight and vigor. *A*, splanchnetomized stomach. *B*, vagotomized stomach. *E*, esophagus. *P*, pyloric portion of stomach. Note the hypertonic condition of stomach *A*.

4. Extirpation of stomachs after section of vagi and splanchnic nerves. Early in the course of the investigation it was observed that the stomachs of splanchnetomized and vagotomized animals exhibited rather wide variations in size, depending on whether the vagi or the splanchnic nerves had been previously severed. This phase of the problem was investigated on ten splanchnetomized and ten vagotomized animals and the stomachs were removed from three to ten days after the operation, directly after the killing of the animals and while the hearts were still

beating. In the selection of these animals care was taken to select frogs of equal size, weight and vigor. The results of these experiments are conclusive in showing that the same general influence which the vagi and splanchnic nerves exert separately on the gastric apparatus may be shown when the splanchnetomized stomach is superimposed upon the vagotomized stomach from two frogs of equal size. The latter or larger stomach represents the atonic and the former or smaller the hypertonic, while the normal stomach takes an intermediate position between the two (fig. 3). It may be said, therefore, that the reciprocal or contrary innervation of Meltzer which may be termed antagonistic tonus, may be physiological as long as it serves the purposes of the organ in question in a beneficial manner. It is pathological as soon as the tonus of one or the other is so exaggerated that the common welfare of the organ is in danger, and that is exactly what happens in the splanchnetomized frog's stomach where the hypertonus of the vagus leads to a state of over-excitability, or to the Eppinger-Hess condition of vagotonia.

PSYCHIC OR REFLEX INHIBITION OF THE GASTRIC HUNGER MOVEMENTS

It was suggested to me by Doctor Rogers early in the course of this investigation that it might be well to study certain cerebral processes in relation to the reflex effects on gastric activity. Previous work on other animals has demonstrated that anything which interests, annoys, frightens or angers, leads to a temporary inhibition of the gastric hunger contractions probably via splanchnics. Furthermore, the sight or smell of food in the dog, at least, leads to this same temporary inhibition if not too often repeated. In order to test further the very important reflex control of the gastric hunger mechanism, as well as of the nervous foci in the medulla, mid-brain and cerebrum concerned in the conduction of sensory and motor hunger impulses, the effects of sound and light stimuli were made use of in the following experiments. The observations were made on six frogs which were later decerebrated and the observations repeated. In the case of the sound stimuli, whistles of different pitches were sounded for periods of from ten to twenty seconds but these caused only very slight gastric inhibition which was of short duration and after two or three repetitions it invariably became ineffective, thus defeating the object of the experiment. Even the filing of a glass rod on the table containing the animal was fully as ineffective in producing inhibition although a second factor must have been intro-

duced, that of vibration. All of these stimuli were of minimal or very moderate intensity and evidently not of sufficient strength to produce an effective and constant reflex, or else the central nervous mechanism for this reflex is at a low degree of development.

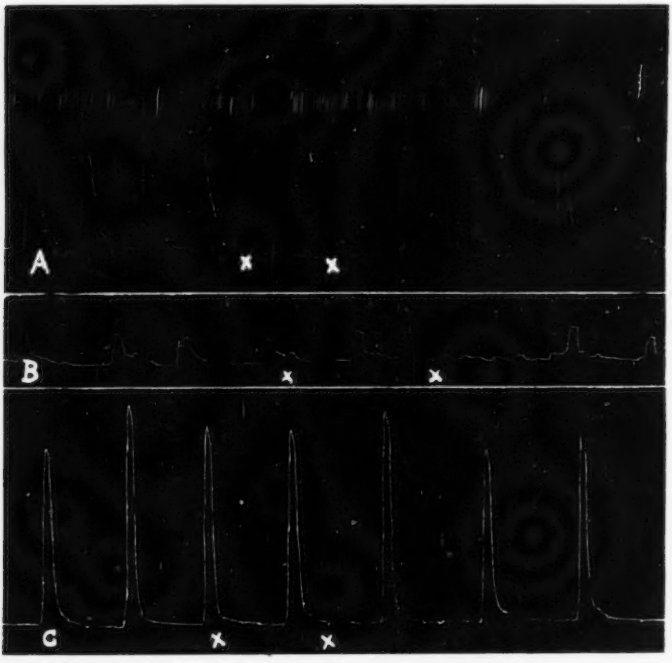

Fig. 4. Records from the empty stomach of frogs. *A*, frog nine days after section of both vagi with splanchnics intact; *B*, frog eight days after section of both splanchnic nerves with vagi intact; *C*, frog nine days after section of the vagi and splanchnic nerves. *x* to *x*, light and darkness shadow test showing temporary inhibition in *A*; very slight inhibition in *B*; and total absence of psychic or reflex inhibition in *C*, in case of the stomach isolated from the central nervous system.

The light stimulus, on the other hand, proved to be more effective. The room was darkened by drawing the shades and while the normal hunger contractions were being recorded the animal was carefully uncovered and the bottom of the window shade directly opposite drawn

back and forth quietly and at a moderate rate, thus casting light and dark shadows upon the animal. This invariably produced temporary inhibition of the movements of the empty stomach and there seemed to be no diminution in the degree of the inhibition after repeated trials. After removal of the cerebral hemispheres there was total absence of the reflex inhibition and the contractions went on uninterrupted. It would appear that the central nervous mechanism for this reflex was more highly developed than that for sound. In other words, from the animal's standpoint it may be considered an important defensive reflex to warn it of its avian enemies as they soar through the air, thus anxiety and fear leading to the characteristic temporary inhibition of the gastric hunger movements.

When the light and darkness shadow test was applied to an animal after complete section of the vagi with the splanchnics intact it invariably led to a temporary inhibition of the gastric hunger movements via splanchnic nerves (fig. 4, *A*). If the same test was applied to an animal after complete section of the splanchnic nerves with the vagi intact it invariably led to only a very slight inhibition of the gastric hunger movements, as represented by a slight and transitory weakening of the contractions (fig. 4, *B*). This slight degree of inhibition usually in evidence after section of the splanchnic nerves is probably due to the action of the few inhibitory fibers in the intact vagi or to some central inhibition of vagus tonus. In the case of the stomach completely isolated from the central nervous system (vagi and splanchnic nerves cut) there is total absence of any psychic or reflex inhibition since the efferent nerve pathways to the stomach have been broken by the sectioning of all the extrinsic nerves (fig. 4, *C*).

CONCLUSIONS

1. Complete isolation of the frog's stomach from the central nervous system leads to hypotonus of the stomach with about the normal type of gastric hunger contractions. This is in confirmation with the work of Carlson on dogs. The automaticity of the gastric mechanism is independent of the extrinsic nerves but these nerves play an important rôle in modifying or regulating the automatic mechanism in the stomach wall.

2. Partial isolation of the stomach from the central nervous system interrupts the normal antagonistic balance between the vagi and splanchnic systems which may lead to pathological reactions such as vagotonia

after complete section of the splanchnic nerves. The stomach in this condition becomes strongly hypertonic while after complete section of the vagi with the splanchnics intact the stomach passes into a hypotonic condition.

3. The acid inhibition of the movements of the empty stomach by stimulation of the gastric mucosa persists after complete isolation of the stomach from the central nervous system, but the inhibition is diminished in intensity and duration. When the splanchnics alone are sectioned the inhibition is even less marked, but after section of the vagi with the splanchnics intact there is, on the whole, a slight augmentation in the inhibition via these nerves. This latter statement is contradictory to the findings of Carlson on dogs, while the other facts above are in accord.

4. The light and darkness shadow test invariably produces psychic or reflex inhibition of the gastric hunger movements in the normal animal. After decerebration or after complete isolation of the stomach from the central nervous system there is total absence of the light reflex on the gastric mechanism. When the vagi alone are sectioned temporary inhibition is the result, while after section of the splanchnics with the vagi intact there is only very slight inhibition produced.

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OBSERVATIONS ON THE RELATION BETWEEN EMOTIONAL AND METABOLIC STABILITY

FREDERICK S. HAMMETT

From the Wistar Institute of Anatomy and Biology, Philadelphia, Pennsylvania

Received for publication June 5, 1920

The studies of Cannon (1) and his co-workers on the effects of the major emotions on the physiological activities of the vegetative nervous system have established and clarified certain fundamental phenomena of general biological importance that are wide-reaching in their application.

While the primary consequences of emotional stimulation have been extensively studied, the secondary involvements such as might be evidenced in the variability of the intermediary metabolism have yet to be studied. The emotional ignition of the vegetative neural complexes can hardly be supposed to be limited to a circumscribed reaction but must indeed reverberate throughout the organism as a whole.

It is obvious that it is possible to divide mankind into two main groups according to temperament or relative emotional stability, between the extremes of which there may exist all gradations of susceptibility to emotional excitation. There are those who are relatively emotionally stable, who pursue the even tenor of their way apparently and actually undisturbed by the surrounding daily happenings. And there are those whose temperaments are of the hair-trigger type, whose emotions are always on tap and who respond to the slightest stimulus with a magnitude of reaction all out of proportion to the value of the stimulus received. Whatever the causes of these differences of susceptibility and response as evidenced in the differences of emotional stability, the results on the recipient and effector organism must of necessity be widely different. That these responses primarily involve the vegetative nervous system has been definitely demonstrated. That they secondarily involve the intermediary metabolism should be expected since the processes of digestion, secretion, absorption, utilization and excretion are all directly or indirectly bound up with the directing or controlling influence of this part of the nervous system.

During the past year opportunity was afforded the writer to study the chemical composition of the blood of emotionally stable and unstable insane and normal persons. The classification of the emotional status of the individuals presented in this study was arrived at by consultation with the physicians in actual contact with the patients and by personal observation over considerable periods of time. The analyses of the bloods were carried out as reported in a previous communication (2) and for the series under discussion here were made at weekly intervals on the seventeen subjects reported. The duration of the periods of observation varied from three to six weeks.

TABLE 1

The coefficient of variability of the blood constituents and the total metabolic variability of the subjects studied

	TOTAL N	NON-PROTEIN N	UREA N	CREATININE N	CREATINE N	URIC ACID N	AMINO ACID N	REST N	SUGAR	TOTAL VARIABILITY
1	7.21	12.81	14.15	5.68	12.54	14.13	7.72	47.78	7.85	129.87
2	5.07	8.09	11.23	6.56	11.15	16.07	32.23	19.19	15.51	125.10
3	11.40	6.84	13.11	8.66	21.60	7.77	16.33	24.07	12.21	121.99
4	11.32	10.73	22.30	5.89	6.56	15.77	16.17	18.02	6.72	113.48
5	11.70	8.19	8.67	3.93	6.96	26.70	4.21	28.13	7.89	106.38
6	8.35	7.71	14.10	4.81	14.82	13.69	10.80	23.42	8.63	106.33
7	10.65	3.99	14.67	4.97	17.50	6.43	6.27	25.54	16.00	106.02
8	2.18	12.86	12.57	7.06	7.00	4.94	11.71	23.48	16.91	98.71
9	9.64	11.33	18.90	6.57	6.26	13.47	12.73	12.16	7.48	98.54
10	3.63	6.22	9.52	5.83	7.32	11.59	17.97	19.19	15.87	97.14
11	9.38	8.87	4.69	1.33	5.54	13.38	5.77	39.72	3.98	92.66
12	1.08	2.19	8.25	4.31	5.90	7.81	3.84	45.03	5.75	84.16
13	3.56	8.78	18.05	4.81	3.64	9.33	3.37	19.47	12.86	83.87
14	10.10	5.22	11.98	3.71	8.80	3.95	17.32	6.06	13.16	80.30
15	9.18	6.96	11.76	6.52	7.28	6.02	10.96	7.50	12.72	78.90
16	8.73	4.18	1.43	12.61	5.74	11.00	10.16	10.84	13.02	77.71
17	17.35	4.98	5.95	4.09	10.06	2.19	8.85	13.58	9.32	76.37

As a basis of correlation between the emotional and metabolic stability, the coefficient of variability (3) for each blood constituent determined for each individual was calculated and the sum of these coefficients was taken as the total variability of the intermediary metabolism of the person in question. Table 1 gives these figures for each constituent determined in each subject. The subjects are arranged in the order of their decreasing variability.

An inspection of the table shows that while the differences in variability of one individual from the next is small, there is a very evident

marked difference between those of highest and of lowest metabolic instability, and this marked difference is correlated with a marked difference in emotional reactivity if we consider the subjects seriatim from the psychological point of view.

Number 1 is a male nurse of the small nervous type, easily upset by minor occurrences and with a continual attitude of worry. Number 2 is a female patient, who although being completely oriented, varies in her emotionalism from deep depression with decreased psycho-motor activity to a wild hilarity and excitement. Number 3 is a male patient, restless, talkative, active and excitable, showing much exhilaration and flightiness. Number 4 is a male patient classed as an agitated depressive and who has firmly fixed somatic and autopsychic delusions. Number 5 is a male nurse, irritable, suspicious and touchy, possessing neither decision nor attention. Number 6, the last of those showing a total variability of over 100, is also a male patient, restless, irritable and excitable, showing considerable emotional elation. The next two members of the series are also of the emotionally unstable type, number 8 being a female patient showing considerable perturbation and violence accompanied by motor activity, screaming, laughing and hallucinations, while number 9 is a male presenting a history of hypersensitiveness, and who becomes apprehensive under examination, is easily depressed and worries, although at other times he is more cheerful. All these individuals so far described, then, can be validly considered as persons of varying emotionalism and of obvious emotional instability. They are also individuals whose metabolic variability is of a relatively high grade, as can be seen from the table. Turning now to the remainder of the subjects the next on the list, no. 10, shows a metabolic variability that is practically the same as found in the latter members of the preceding group. Yet his emotional status to all appearances is one of relative stability as far as can be determined. He is pleasant and agreeable and inclined to be seclusive. He is quiet and not irritable. An inspection of the figures obtained for the metabolic stability from now on shows not only gradually decreasing values but also values definitely and markedly lower, as a group, from those preceding. Number 11, a male patient, is quiet and sits as though in deep thought. He is of rather an even temperament, occasionally becomes angry, but is usually able to control himself. Number 12 is a male patient, quiet, maintaining a given posture for some length of time, sits rigidly in a chair and stares vacantly into space. He is indifferent toward his surroundings. Number 13 is a female patient

who is emotionally apathetic and indifferent. Number 14, a male patient, is at times excitable but is only apparently superficially disturbed since he eats regularly and well. Number 15, a male patient, was in a catatonic stupor throughout the period of observation and obviously was not emotionally variable, to any determinable extent. Number 16, another male patient, is never excited but is always indifferent, apathetic and seclusive. Number 17, the last of the series, is a male nurse, the emotional antithesis of number 1. He is phlegmatic and inexcitable, paying no attention to the ordinary little vicissitudes of life.

From the psychological point of view it is evident that these latter individuals present the appearance of being relatively emotionally stable. As a group they are generally inexcitable and are not roused to demonstrable emotional reactions by circumstances which act as stimuli causing the marked response of the first group of high metabolic variability.

This relation between a relatively high metabolic stability and a low grade of emotional reaction, and between a relatively low metabolic stability and a condition of temperamental excitability is by no means claimed to be exact or quantitative. Nevertheless the data seem to indicate such a tendency.

The logical conclusion to be drawn from this comparison is that larger variations in intermediary metabolism are prone to accompany conditions of ready emotional response of a marked nature to disturbing stimulation, and that on the other hand the variability of the intermediary metabolism in individuals who are less susceptible is liable to be relatively low.

A broader application of the tendency here demonstrated can be made if for a moment one compares the physical condition of the so-called emotional type of individual with his more phlegmatic and less responsive emotional opposite. The former usually presents a picture of deficient nutrition, the latter is in most cases well supplied with the anabolic products of metabolism. The metabolism of the one by its wide variability gives indications of the possibility of there being at one time an overtaxing of the organism, and at another time of the organism lacking a sufficient energy supply. The other type is relatively more metabolically uniform. His metabolism consists of a balanced give and take, in which no undue strain is put on the catabolic processes nor is there a lack of sufficient material to supply the anabolic needs. The results of these processes are shown in the end

products, as indicated by the figures for variability here presented, and the causes are fairly attributable, other factors being absent, to the relative magnitude and type of emotional response of the individual to the incident stimuli.

Such a conception while lacking complete demonstration is nevertheless supported by correlated observations and fits in not only with the observations recorded here but also with the general hypotheses of Cannon (1) as to the susceptibility of the organism to respond to the effects of emotional stimuli by disturbances of the vegetative nervous system.

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FOUR FACTORS CAUSING CHANGES IN THE TYPE OF RESPONSE OF THE ISOLATED INTESTINAL SEGMENT OF THE ALBINO RAT (MUS NORVEGICUS ALBINUS) TO SODIUM CARBONATE

S. HATAI AND F. S. HAMMETT

From the Wistar Institute of Anatomy and Biology, Philadelphia, Pennsylvania

Received for publication June 12, 1920

The usual response of the isolated duodenal segment from the albino rat intestine is that of shortening or contraction when stimulated by the application of weak solutions of sodium carbonate. Occasionally however segments are encountered that exhibit irregularities in the type of response. One may fail to react to the stimulating substance, another may answer by a preliminary slight contraction which is followed by a relaxation below the original tone level from where the response was elicited, while a third may undergo a prompt and decided relaxation. This last is the irregularity most frequently encountered.

This occasional inconstancy in reaction of segments from apparently normal animals which showed itself at times as a complete reversal of the type of response demanded investigation because it was necessary for our purposes to have rats on hand the intestinal segments of which could be relied upon to give uniform and consistent reactions to stimulation by weak sodium carbonate solutions.

The inconstant responses could not be attributed to variations in the technique of preparation of the segments nor to the carrying out of the tests since this was always uniform. The control of the rats up to the time of killing with respect to age, sex, health, heredity and diet was also in the hands of the authors and consequently these factors were regulated. The preliminary observations which gave satisfactory results had been made on rats which had been for some time in the laboratory cages. Irregularities first began to appear when animals were used before they had become accustomed to the laboratory cages.

Donaldson (1) having reported that the domesticated albino rat is extremely sensitive to changes in environment, we began to think that possibly the change from the colony house to the laboratory had in-

duced an excitement causing the variations. This idea that excitement was the cause of the irregularities was considerably fortified when the segment taken from a rat which had resisted capture and had become quite agitated gave a marked relaxation on stimulation by carbonate. The test of this hypothesis was easy.

The method of procedure for the preparation of the segments for testing was as follows. The rat was first put under light ether anesthesia and then killed by crushing the cord in the cervical region. The duodenal portion of the intestinal tract was then removed without stretching, and cleaned of mesenteric fat. A segment about 1.5 cm. in length was cut from the gastric end of the duodenum and suspended by silk threads in a glass cell containing 4 cc. of Tyrode's solution kept at body temperature and through which oxygen was continually passing. One end of the segment was attached by a thread to a support within the cell and the other end was connected with a light lever writing on a slowly moving drum. Rhythmical contractions immediately appeared and were recorded for one revolution of the drum (about 10 minutes) by which time the tone level had become uniform and consistently parallel with the base line.

With segments prepared in this way the addition of 0.25 or 0.50 cc. of an M/10 solution of sodium carbonate to the Tyrode's solution in the cell usually caused a shortening of the segment with a consequent rise of the curve of rhythmical contractions as is shown in the tracings. It was possible to obtain this type of reaction from one and the same segment for several successive applications of the carbonate. After each application the cell and segment were thoroughly washed with oxygenated Tyrode's solution kept at body temperature. After washing the segment as a rule came back to the original tone level within one revolution of the drum and was then ready for another test. The tests as presented in figures 1 to 5 were obtained in this manner.

In order to determine whether the excitement of the rat brought about either by change of environment or by other means was a factor in causing the irregularities previously described, a number of male rats of the same age and on the same diet were brought to the laboratory cages and allowed to become accustomed to the new environment during three or four days. When a segment from one of the lot was prepared as usual and tracings of the effect of the sodium carbonate stimulation recorded the normal reaction was invariably obtained. When others of the same lot were annoyed by various methods just before killing and similar tracings made the irregularity of reaction occurred

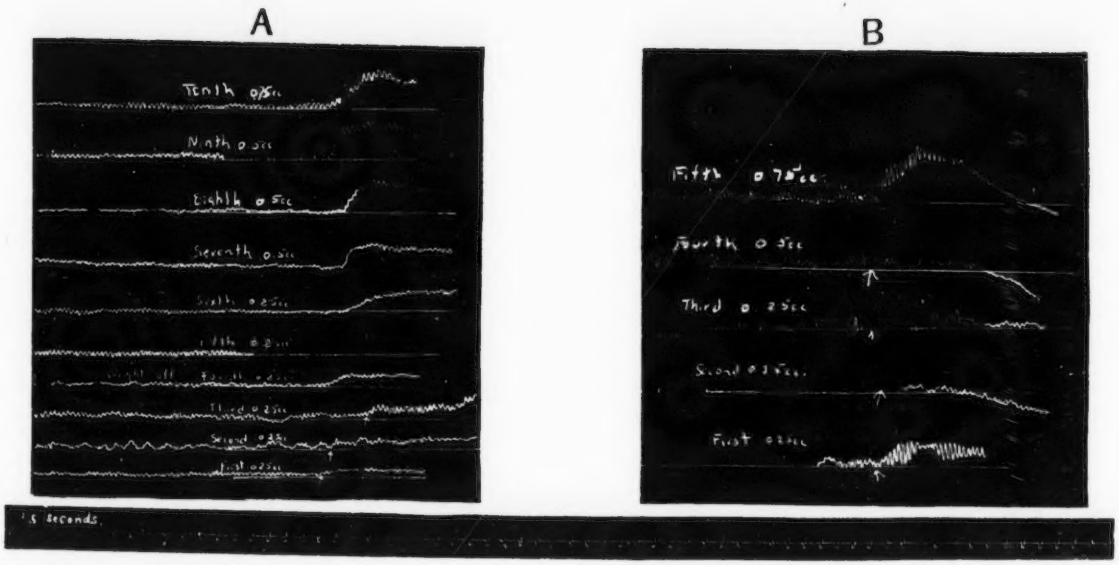

Fig. 1. *A*. Undisturbed normal male rat. Age 100 days. *B*. Disturbed normal male rat. Age 100 days.

in practically every case and usually took the form of a reversal of the type of response as shown in figure 1. Occasionally however an animal was found which did not appear to be susceptible to the preliminary excitation. Such an one is shown in figure 2, rat *C*. Nevertheless the relative frequency of the irregularity was such as to establish the hypothesis that excitement is one of the factors causing variation in the response of the isolated intestinal segment to sodium carbonate stimulation. In this connection it should be noted that although relaxation occurs in the segments from excited animals, this type of response gives way to the normal type after several applications of the sodium carbonate.

The results described in the preceding paragraphs were obtained from rats close to one hundred days old. Desirous of not limiting our material to animals of this age, we attempted to continue the observations on rats one hundred and fifty days old. Here it was found that while evidences of irregularities were present in rhythm, amplitude and base line level, the type of response was usually a normal contraction. When we used a series of rats some two hundred days old it was found that these older animals were quite insusceptible to the effects of the preliminary excitation as indicated by the response of the duodenal segment to carbonate stimulation. This is plainly shown in figure 3. There were no exceptions. It is accordingly evident that the disturbing effect of excitement is modified by age and that immaturity is one of the factors causing susceptibility to excitement with resulting irregularities of response.

The exclusive use of male rats has certain obvious disadvantages. With this point in mind several series of tests were made using as controls undisturbed males and comparing with them females of like age and conditions of environment and diet. It was found that when female rats were used the response of the segment to the sodium carbonate stimulation was normal if the animals were not menstruating. When menstruating, however, as evidenced by congestion of the uterus, variability of response was uniformly obtained and of the same type as that given by segments from the young disturbed male rats. This is shown in figure 4. Hence menstruation is a factor preventing the uniform response to carbonate stimulation and the test demonstrates that female rats during sexual activity are not suitable material for general use.

The observations up to this point contribute further support to Cannon's (2) theory of the influence of emotional reactions on the vegetative

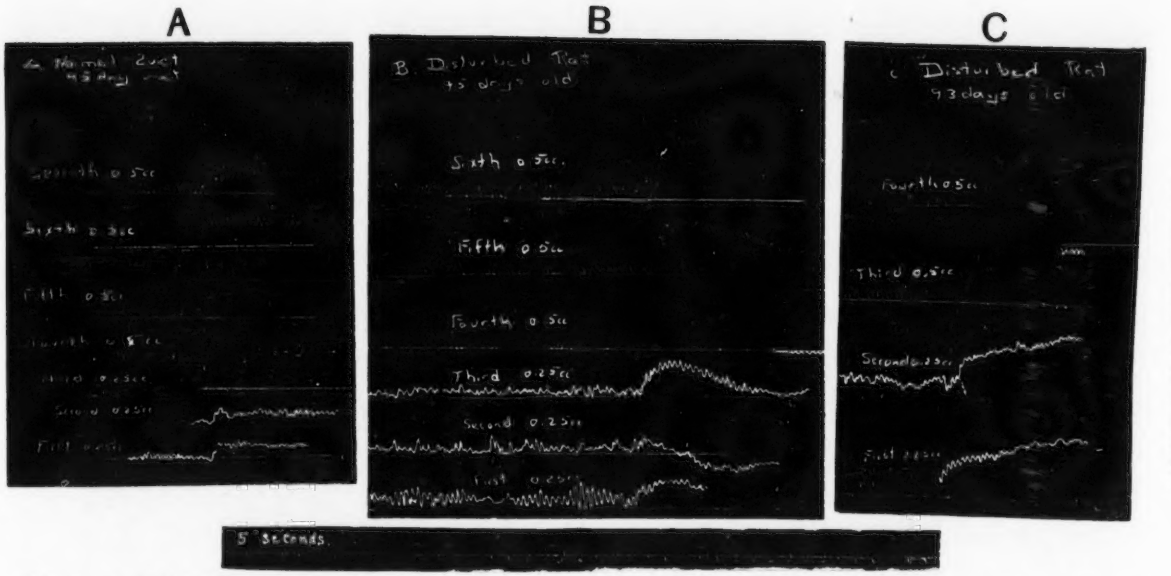

Fig. 2. *A*. Undisturbed normal male rat. Age 95 days. *B*. Disturbed normal male rat. Age 95 days. *C*. Disturbed normal male rat. Age 93 days.

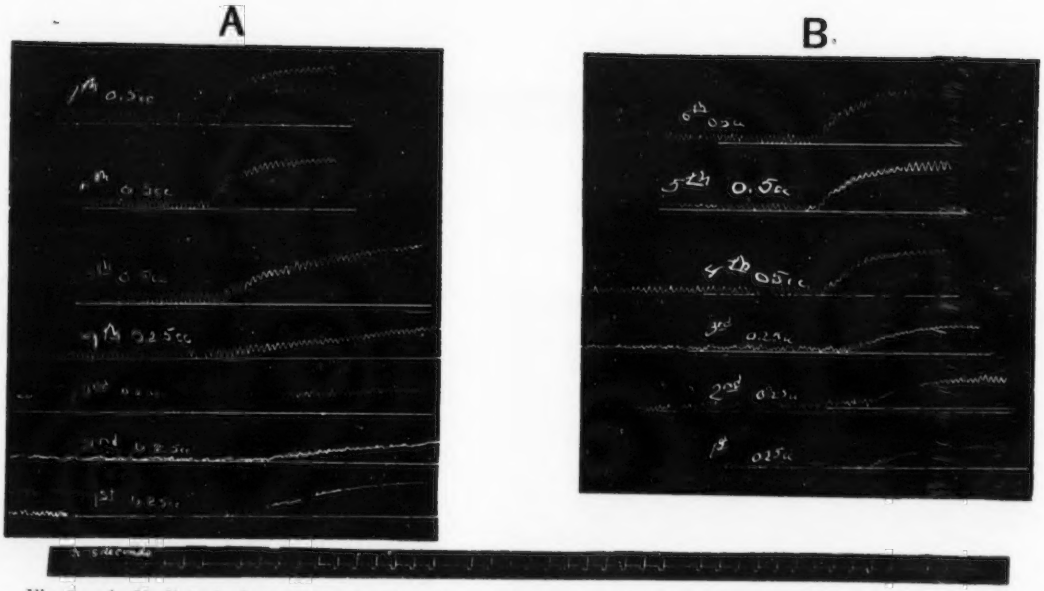

Fig. 3. *A*. Undisturbed normal male rat. Age 200 days. *B*. Disturbed normal male rat. Age 200 days.

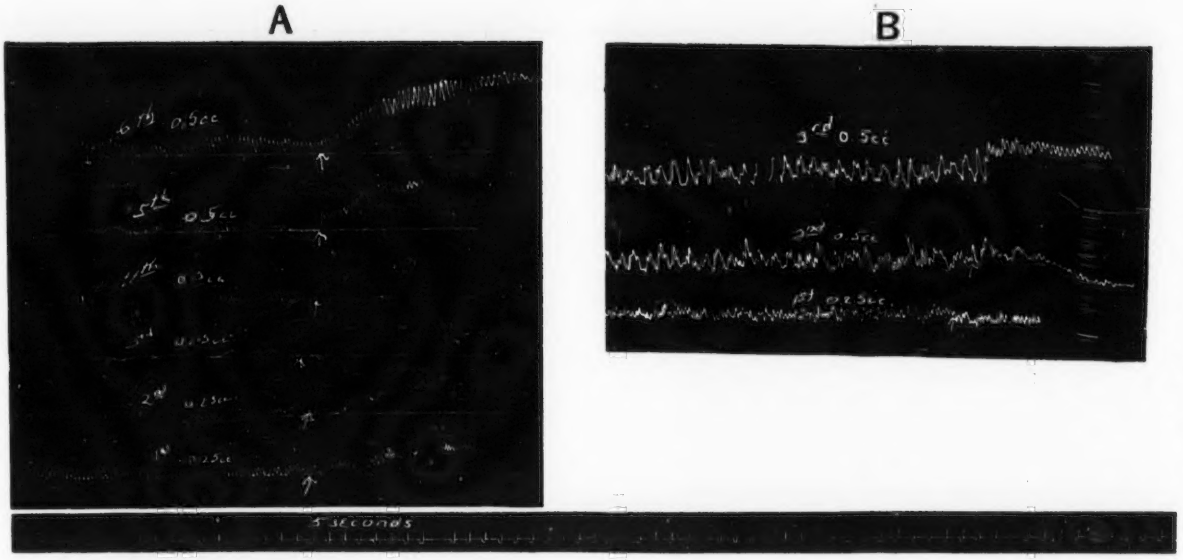

Fig. 4. *A*. Undisturbed normal male rat. Age 200 days. *B*. Undisturbed normal female rat in heat. Age 75 days.

system. They also bear out with more exactness the observation made by Alvarez (3) that the intestine of younger animals (rabbits in his experiments) tends to be more unstable than does that of the older.

It seemed desirable to determine the immediate cause of these phenomena. In view of Cannon's (2) studies, the conception of an endocrine origin of the differences has a certain plausibility. Against this notion is our observation that we were unable to obtain any but a normal response to carbonate stimulation from the segment from the normal animal after application *in vitro* of extracts of the homologous adrenals, hypophysis or thyroid. The dependence of the normal mode of movement of the intestine on the integrity of Auerbach's plexus as demonstrated by Magnus (4) and the hypothesis that emotional disturbances spread to the vegetative system, in part at least, through the splanchnics, led us to study the effect of stimulating the superior splanchnic. The rats used were all males of the same age and living in the same conditions. The tracings obtained are given in figure 5. The tracing A was made by the isolated segment of an undisurbed rat when stimulated by M/10 sodium carbonate. It is normal. The tracing B was made by the segment from a rat that had been annoyed and shows the characteristic effect of excitement on the response. Tracing C was made bt the segment from an undisturbed rat which had been opened immediately after killing and the splanchnic nerve electrically stimulated before removal of the segment. It is quite evident that in this case the preliminary splanchnic excitation has induced changes in the intestine of a nature that has caused the segment to respond as do segments obtained from young emotionally excited males. Splanchnic irritation is therefore a fourth factor in causing irregularity in response to sodium carbonate stimulation. The unusual height to which the curve rises is noteworthy although no explanation can be offered for the intense response after the preliminary relaxations. This relaxation on the application of the sodium carbonate solution to the duodenal segment isolated after splanchnic stimulation is similar in type to the relaxation of the intestinal segment of the intact animal after splanchnic stimulation alone, as obtained by Bayliss and Starling (5), and is very suggestive.

Of the four factors so far investigated it seems as if the last revealed the path for the expression of the other three. There is at present no reason to suppose that the splanchnic stimulation could so affect the adrenals as to cause a secondary reaction through the mediation of adrenalin on the intestinal segment since there was no vascular circula-

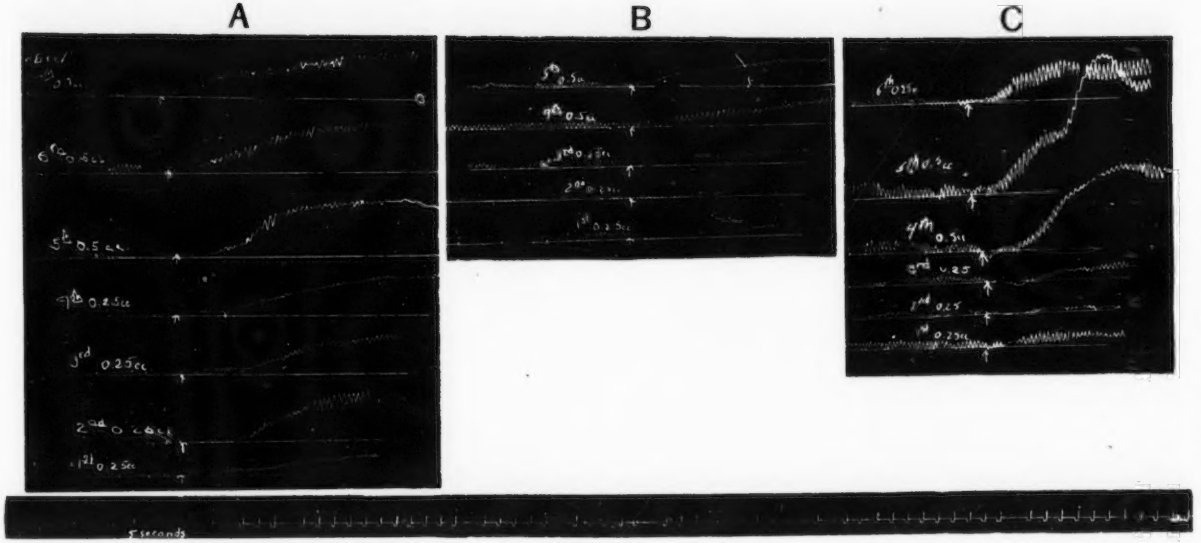

Fig. 5. *A*. Undisturbed normal male rat. Age 80 days. *B*. Disturbed normal male rat. Age 80 days. *C*. Normal male rat electric splanchnic stimulation. Age 80 days.

tion at the time stimulation began. And further, we showed earlier that adrenalin did not modify the response to sodium carbonate. Consequently we are inclined to the opinion that the irregularities of response observed are due to disturbances of the intestinal plexus.

SUMMARY

These experiments demonstrate that the normal response of an isolated duodenal segment of the albino rat to sodium carbonate stimulation is one of shortening or contraction but that certain factors may modify the type of response.

These irregularities of response of the segments from young, healthy male rats are due to a condition of excitement induced in the animal by change of environment or rough handling just before its use for experimental purposes.

The susceptibility of the intestinal segment to such external disturbances is modified by age in that material from rats of two hundred days fails to show the irregularities after excitation.

Female rats are not suitable subjects for general studies of this nature inasmuch as the act of menstruation sets up such changes in the intestinal segment as to cause it to respond in a manner analogous to the segment from young excited male rats.

The electrical stimulation of the splanchnic nerve of a normal undisturbed male rat before the removal of the segment results in the production of a similar type of response to carbonate stimulation as is that obtained from the segment of the excited rat of the same age. This inclines us toward the hypothesis that the irregularities of response here observed are due to disturbances of the intestinal plexus.

CONCLUSION

It is possible to obtain an intestinal segment which will give a uniform and consistent response to sodium carbonate stimulation. The gastric end of a duodenal segment satisfies these requirements when taken from healthy male adult albino rats eighty to two hundred days old some fifteen hours after the last feeding and in which no emotional disturbance has been induced by recent changes of environment or rough handling. Excitement, age, menstruation and electric stimulation of the splanchnic nerves are factors tending to cause changes in the type of response of the segment to sodium carbonate.

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THE ADJUSTMENT OF BLOOD VOLUME AFTER INJECTION OF ISOTONIC SOLUTIONS OF VARIED COMPOSITION[1]

ARTHUR H. SMITH AND LAFAYETTE B. MENDEL

From the Sheffield Laboratory of Physiological Chemistry, Yale University, New Haven

Received for publication June 12, 1920

Water plays such a large part in the physical regulation of fundamental physiological processes as well as in the chemical transformations in the body, that the lack of suitable amounts or the abnormal distribution of fluid in the body may be correlated with distinctly pathological conditions. Volume changes in the body take place only through the movement of water.

The various types of edema illustrate what may happen when there is an abnormal distribution of water in the body. Besides the edema associated with cardiac and renal diseases we may add another type known as nutritional edema. The etiology of neither of these conditions is clear.

There are also conditions in which water is lost from the body to such an extent that desiccation takes place and the blood becomes exceedingly concentrated. Such a condition prevails in Asiatic cholera in fatal cases of which Rogers (1) has reported a serum loss of 62 per cent. Intravenous injection of isotonic saline solution in such cases is at once followed by diarrhea with the resulting loss of all the added water. In war gas poisoning Underhill (2) has described the marked loss of water from the blood and the movement of water into the lungs.

The mechanism of the loss of heat from the body is largely physical in character. Balcar, Sansum and Woodyatt (3) have reported experiments in which they were able to produce fever at will by intravenous injections of hypertonic glucose solutions provided diuresis ensued. Temperatures of 109°, 111° and 126° were thus obtained in dogs. These authors have suggested that in the body the water may be either "free" or "combined." The "free" water is available for evaporation

[1] The data in this paper are taken from a dissertation presented by Arthur H. Smith for the degree of Doctor of Philosophy, Yale University, 1920.

with the consequent heat loss while the water combined with the colloids of the body is not available for heat regulation. Any condition which increases the "combined" water or decreases the "free" water leads to a rise in temperature due to the absence of proper heat loss. Lussky and Friedstein (4) reported that in cases of pneumonia ending in crisis in infants, there was a decrease in body weight with crisis due to loss of water.

The large amount of water in the cell may aid considerably in maintaining the optimum temperature of the cell, for water has a high specific heat. The large percentage of water in tissues in which oxidation is most intense, may be correlated with this unique property of acting as a heat buffer.

Another evidence of water movement in the body has recently been reported by Weed and McKibben (5). They found that the brain increased in volume after injection of hypotonic salt solutions or distilled water and decreased in volume after hypertonic solutions—these changes being independent of vascular changes and of the cerebrospinal fluid pressure. The remarkable clinical study of Cushing (6) on the alteration of brain volume after administration of hypertonic salt solution per os indicates that the brain is peculiarly sensitive to the movement of water in the tissues.

The question naturally arises as to the physical character of water in the body. By drying blood one finds that it contains nearly 81 per cent of water. But is this water in the form with which we are familiar? In health some of this water is in such a form that it can be given off as vapor in the lungs and can be appropriated by the sweat glands and given off as water. When one considers, however, that in the blood and in the cells there is really a solution of protein and lipoid in aqueous solution of salts, it becomes apparent at once that the physical chemical relationships of water to the other components of the body fluids are exceedingly complex. The hydrophilous colloids probably take up water by adsorption on the internal or disperse phase. This adsorbed water then dissolves in the substance of the particles of the colloid. Large amounts of water are thus taken up by gums, proteins and other emulsoid colloids with the result that swelling takes place.

This imbibition of water is dependent on a variety of conditions such as hydrogen ion concentration and nature of the salt present. It is to the study of some of these conditions on the passage of water out of the circulation that the present investigation is devoted.

In the study of the application of physical laws to the behavior of colloids, much importance has been attached to the early work of Hofmeister and his pupils. Lewith (7) studied the efficiency of various ions in precipitating the serum proteins. The arrangement of his series in the ability to precipitate the proteins was as follows: nitrate<chloride<acetate<sulfate. Hofmeister (8) studied the comparative efficiency of anions in salting out egg globulin and obtained the following series: sulfate>phosphate>acetate>citrate>tartrate>bicarbonate>chromate>nitrate>chlorate. In a later series of experiments (9) he found that the order of efficiency in precipitating gelatin was sulfate>citrate>tartrate>acetate>chloride>nitrate>chlorate. For the precipitation of colloidal iron the series was practically the same. When he studied the swelling of gelatin discs in various solutions, Hofmeister (10) obtained the following order of efficiency: sulfate, tartrate, citrate<acetate<chloride<chlorate, nitrate, bromide, in prompting the swelling of the discs.

Pauli (11) found that different salts raised the coagulation temperature of proteins to different degrees and the order of efficiency was citrate, sulfate<chloride<iodide<acetate<chlorate<nitrate<bromide. Lillie (12) reported that the order of salts in decreasing the osmotic pressure of gelatin and egg albumen was sulfate, tartrate, citrate, phosphate, ferricyanide>fluoride, chloride, nitrate, chlorate>bromide>iodide>sulfocyanate.

Studies of the same series of salts applied to "living" colloids, i.e., to the protoplasm or membrane of cells, have in many cases shown a somewhat similar order of activity. Höber (13) found that the order of anions for stimulating frog muscle was sulfate<chloride<nitrate<bromide<iodide<sulfocyanate. Likewise the order of efficiency of the anions in hemolyzing blood cells in slightly hypertonic solution was sulfate<chloride<bromide<nitrate<iodide. Lillie (14) has pointed out that those salts that are good activators for Arbacia eggs, increase the surface permeability of the egg and the order of efficiency of the salts is chloride<bromide<nitrate<sulfocyanate<iodide. Höber (15) studied the absorption of salts from the small intestine. Sodium chloride was absorbed faster than sodium sulfate and for the series he found chloride>bromide>iodide>sulfate in speed of absorption.

Reviewing the evidence it seems that the sulfate, citrate and tartrate tend always to decrease the permeability, to increase the precipitability of protein, to lessen the imbibition of water, while chloride and acetate assume a middle position and nitrate, bromide, iodide and sulfocyanate tend to increase the permeability, to increase the imbibition of water and to lessen the precipitability of protein. In other words, as Spaeth (16) has pointed out, the order of dispersion of hydrophilous colloids by anions is sulfate<tartrate<citrate<acetate<chloride<chlorate<bromide<iodide<sulfocyanate which is the same order in which these ions produce their deleterious effect on cells.

The method employed in the following series of experiments consisted in the sudden increase of the blood volume to twice the normal volume and the measure of subsequent volume changes by means of hemoglobin determinations. The injected fluid was an isotonic solution of the salt under consideration or, if the toxicity of the substance prohibited, the maximum non-lethal dose made isotonic with sodium chloride.

Healthy rabbits in good nutritive condition were used in all the experiments. The solutions were isotonic with rabbit blood, the osmotic pressure being controlled by freezing point determinations. They were kept at the proper temperature in thermos bottles and injected at 37°C. The injection was made during ether anesthesia through a cannula into the left jugular vein from a graduated cylinder by means of pressure at such a rate that in two minutes a volume of fluid equal to the estimated blood volume of the animal was introduced. In some cases second and third injections were made. In the early experiments the figure 50 cc. per kilo (cf. Boycott, 17) was used as the normal blood volume of the rabbit but later the value $\frac{1}{19.1}$ of the body weight was used.

From a cannula in the right carotid, samples of blood were taken before injection, immediately after injection and thereafter at five-minute intervals for one half hour followed by ten-minute intervals until the end of the experiment. The few drops of blood required for the analysis were caught in depressions of a paraffin plate from which measured volumes were immediately taken for the hemoglobin determinations. In the early part of the work these were made by the method of Haldane (18) using a standard whose oxygen capacity was determined by the method of Barcroft (19). The hemoglobin determinations made later were done by the method of Cohen and Smith (20).

The bladder of the rabbit was emptied by squeezing before the experiment and all of the urine voided during the experiment and that remaining in the bladder on autopsy was collected and measured. At the conclusion of the experiment the animal was killed and autopsy made.

The term relative blood volume is used to mean the ratio of the hemoglobin percentage after the injection to that before the injection. The method of calculating it is as follows: assuming the original volume to be 100, if the hemoglobin value before the injection is 80 and after it is 50, the relative blood volume after injection is $\frac{80}{50} \times 100 = 160$. These figures for relative blood volume, then, are really the percentage of the normal blood volume based on the hemoglobin content.

The use of hemoglobin determinations as an index to the blood volume changes requires special consideration. This is a valid index only if, under the conditions of the experiment, there is a negligible variation in the erythrocyte count due to extrusion of new red cells into the blood stream. Increases in the red count and hemoglobin have been reported in a variety of conditions. Muscular contrac-

tion (21), exercise with perspiration (22), increase in blood pressure (23), and decrease in atmospheric pressure (24), have all been reported as conditions under which there is an increased red count and hemoglobin. Lamson (25) has investigated the factors producing polycythemia. He found that in some cases hemorrhage will produce an increased blood count shortly after the blood is lost.

TABLE 1

Comparison of variation in hemoglobin with that of total solids before and after injection of isotonic salt solution

	BEFORE INJECTION	AFTER INJECTION	REDUCTION
	per cent	*per cent*	*per cent*
Experiment 30			
Hemoglobin	90.0	73.0	81.0
Solids	16.0	13.3	83.0
Chlorides of whole blood	0.42	0.36	86.0
Experiment 46			
Hemoglobin	67.0	56.0	16.4
Solids	13.8	11.9	13.4
Experiment 48			
Hemoglobin	75.0	64.0	14.6
Solids	14.7	12.9	12.2

TABLE 2

The effect of repeated small hemorrhages on hemoglobin percentage

TIME INTERVAL FOR REMOVAL OF SAMPLES CA. 1–2 CC.	EXPERIMENT NUMBER		
	40	50	51
minutes			
Normal	67	89	65
5	65	90	65
10	62	90	65
15	60	90	66
20	59	90	65
25	59	90	66
30	59	90	67
40	59	89	66
50	59	90	65
60	59	89	65

Among other factors he mentions emotional excitement and anesthesia. If these factors were to play a part in our experiments the validity of the results obtained might be questioned.

Lamson quotes figures illustrating the increase in red count in a cat after fright. The degree of polycythemia is exceedingly variable. He also states that immedi-

ately after anesthesia with ether the red count is above normal but that it gradually falls. It is conceivable that there may be an increase in red count without an increase in hemoglobin.

Experiments were carried out to determine whether or not the hemoglobin could be used as an index to blood volume under the conditions of the procedure outlined above and also whether the hemoglobin percentage changed as a result of excitement and anesthesia in these experiments. The results (tables 1 and 2) show that not only did anesthesia, fright and withdrawal of successive small volumes of blood not change the hemoglobin percentage, but that the hemoglobin percentage varied in a parallel manner with the total solids. It seems reasonably certain, then, that under the experimental conditions outlined, the hemoglobin is a valid and useful index to blood volume changes.

Inasmuch as glycosuria has been observed following intravenous injections of salt solutions, it seemed of importance to determine whether or not there was hyperglycemia which, of itself, might exert some influence on the dilution of the blood. In the present experiments there was no glycosuria following the injections except with colloidal silver. In one experiment (exper. 24) when sodium sulfocyanate was injected, the blood sugar was 0.075 per cent before anesthesia, 0.112 per cent after anesthesia and 0.209 per cent one half hour after the injection. At this time the relative blood volume had returned to normal so that it may be said that such hyperglycemia as follows intravenous injection of salt solutions, exerts no appreciable effect on blood volume. This point needs, however, further investigation.

Experiments with salt solutions. The following solutions were used: Sodium chloride 0.98 per cent, sodium acetate 1.23 per cent, sodium bromide, 1.8 per cent, sodium nitrate 1.5 per cent, sodium sulfocyanate 1.36 per cent, sodium sulfate 2.0 per cent, sodium tartrate 1.5 per cent in 0.45 per cent sodium chloride and sodium citrate 0.26 per cent in 0.9 per cent sodium chloride. These solutions were isotonic with rabbits blood. The injection was rapid—a volume equal to the blood volume being introduced in two minutes. In the case of the chloride, bromide and sulfate in some of the experiments, the respiration was slower and more labored during the injection. In the case of tartrate the respiration was quickened. With the citrate there were muscular spasms.

From table 3 it will be seen that the salts differ somewhat in their influence on the passage of water out of the circulation. With citrate, tartrate and sulfate the relative blood volume stayed above normal longer than in any other case; with the chloride the return to normal

was more rapid while with the bromide, acetate and nitrate the return to normal blood volume was most rapid. Except in the case of the citrate, tartrate and sulfate, however, the differences were negligible.

In all of these experiments the larger part of the injected fluid disappears within the first five minutes and a considerable part of it leaves the circulation during the injection (17). Immediately after the injection there is an enormous increase in the filtration pressure in the capillaries. This increased pressure accounts for the rough parallelism in volume changes between all the salts within the first five minutes after injection.

TABLE 3

Regulation of blood volume after injection of solutions of various sodium salts; average results of all experiments; relative blood volume

TIME INTERVAL	SODIUM SALT							
	Acetate	Nitrate	Sulfo-cyanate	Bromide	Chloride	Tartrate	Sulfate	Titrate
minutes								
Normal	100	100	100	100	100	100	100	100
Immediately	164	166	158	142	141	148	148	137
5	116	130	128	119	118	123	124	119
10	108	118	117	111	114	118	116	120
15	104	108	111	109	112	115	112	116
20	104	108	107	105	109	110	112	115
25	104	106	106	104	106	110		114
30	101	103	104	104	105	111	112	114
40	100	100	102	101	102	111	112	114
50		100	102		102	111	109	
60							107	

The differences in behavior between the citrate, tartrate and sulfate and the rest of the salts became apparent later in the experiment, i.e., twenty-five or thirty minutes after the injection. The effect of the given salt on the diffusion of fluid is then seen. On the basis of the present experiments the salts can be arranged in the order of their effect in permitting the passage of fluid out of the circulation, as follows: —acetate, nitrate, sulfocyanate, bromide > chloride > tartrate, sulfate, citrate.

The physical chemical systems in the blood vessels immediately after introducing the injection fluid are very complex. In the blood the plasma proteins, the red corpuscles and lipoids occur suspended in, or dissolved in, a water solution of various inorganic salts. It is obvious

that the injection fluid will first react with the substances in the blood insofar as it is capable of producing any change in the equilibrium there. Since proteins are ionized and obey the law of mass action (26), (27) a chemical readjustment in the blood stream after the injection of the salt solutions may be expected. Hence the character of the fluid thus established in this newly constituted system may be sufficient to account for the differences of rate with which fluid leaves the circulation after the injections of various salt solutions.

Again, although we were very careful to have the solutions isotonic when injected, it does not follow that the osmotic conditions in the blood stream remain unchanged after the addition of the salts. There may have been some degree of ion-colloid readjustment or even double decomposition between inorganic salts which might change the osmotic pressure of the blood temporarily. The calcium precipitants, especially, might act in this way.

A third possible factor in regulating the speed with which the added fluid leaves the circulation is the "permeability" of the membrane forming the capillary wall. It is obvious that most of the investigations of the permeability of isolated plant and animal cells can not be applied to the capillary endothelial membrane except in a very general way, for the proteins and especially the salts of the plasma constitute, as can be inferred from the above discussion, a very effective system for maintaining not only a constant reaction but also a constant balance of salts and colloids. It is because of this regulating mechanism that the difference between the salts used in these experiments was so small when judged by their effect on the movement of fluid out of the circulation. The sulfate, tartrate and citrate action was more pronounced because of their double effect: they may have reduced the water-holding capacity of serum proteins either through formation of less ionized combinations or through purely physical means; and secondly, they may have decreased the permeability of the capillary membranes so that the free movement of surplus fluid out of the circulation observed with the chloride, for instance, was interfered with. It is possible that the combination of the above mentioned factors resulted in the diminished speed of return of the augmented blood volume observed after injection of solutions containing sulfate, tartrate or citrate.

The analogy between the action of the various salts on the capillary membranes and on other membranes is suggestive. Magnesium sulfate, sodium sulfate, magnesium citrate and sodium potassium tartrate, saline cathartics containing anions which appear at one end of our series

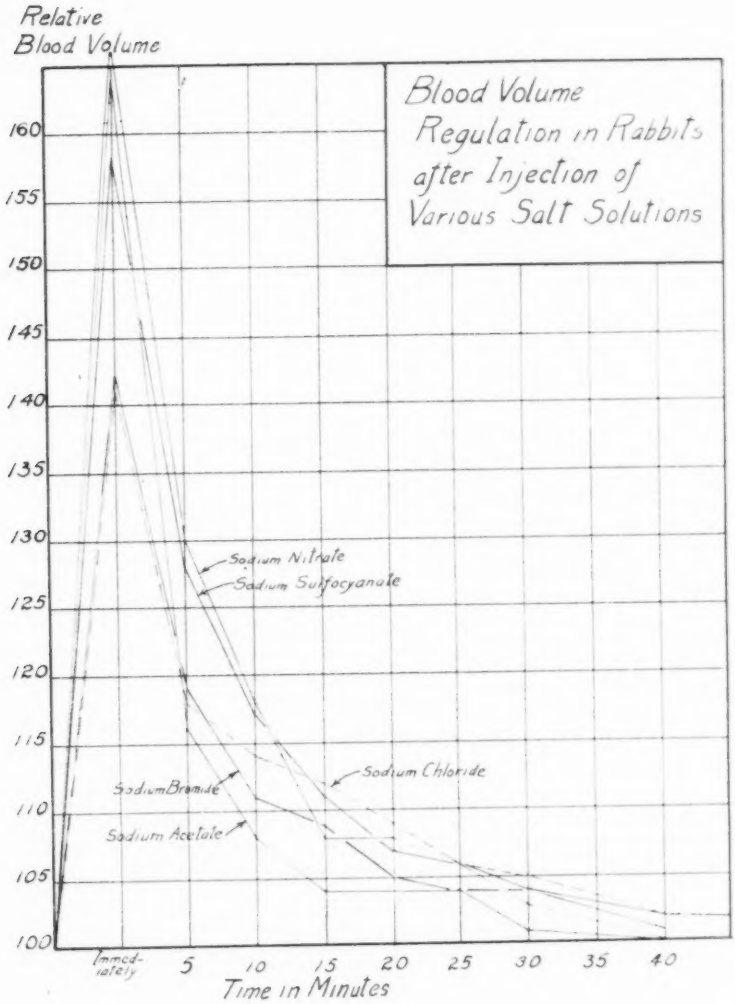
Relative
Blood Volume
Blood Volume
Regulation in Rabbits
after Injection of
Various Salt Solutions
160
155
150
145
140
135
130
125
120
115
110
105
100
Sodium Nitrate
Sodium Sulfocyanate
Sodium Chloride
Sodium Bromide
Sodium Acetate
Immed-
iately
5
10
15
20
25
30
35
40
Time in Minutes

Chart 1

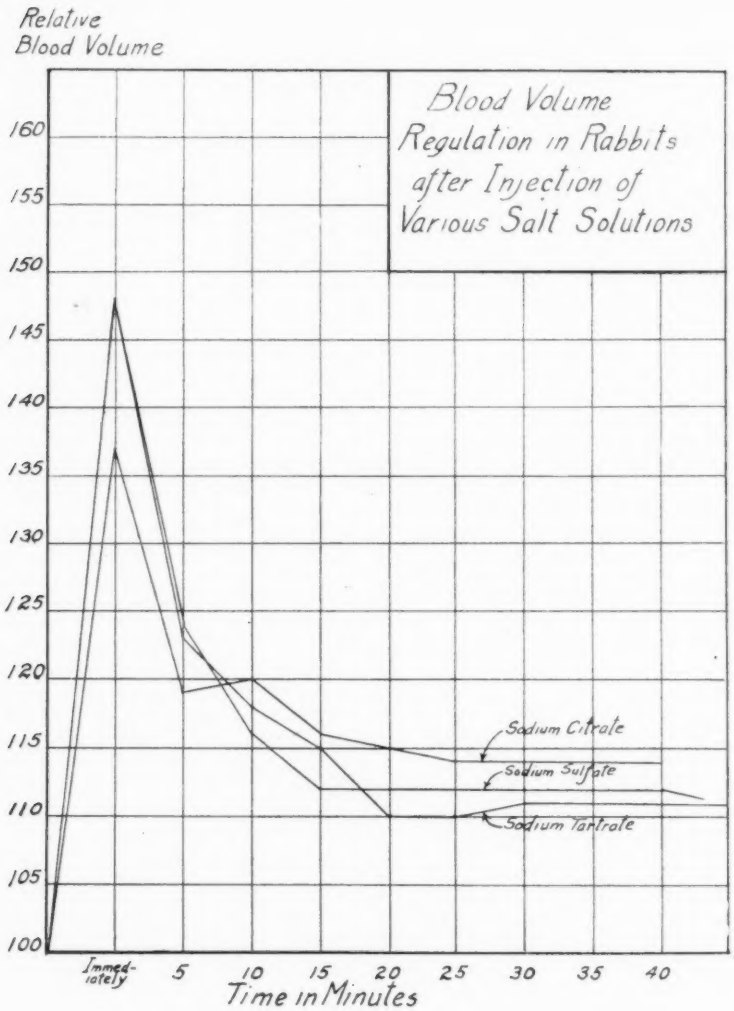
Relative
Blood Volume
Blood Volume
Regulation in Rabbits
after Injection of
Various Salt Solutions
160
155
150
145
140
135
130
125
120
115
110
105
100
Sodium Citrate
Sodium Sulfate
Sodium Tartrate
Immed-
iately
5
10
15
20
25
30
35
40
Time in Minutes

Chart 2

do not easily diffuse through the gut wall into the blood stream, and so they act osmotically to draw water from the blood stream to flush the intestine. Goldschmidt and Dayton (28) have shown that this is precisely what happens in the colon. There was free passage of water with practically no diffusion of sulfate in their experiments. Hober (15) has studied the absorption of salts from the small intestine and finds that the anions of the salts arrange themselves in the same order as in the present experiments, sulfate being absorbed least rapidly and chloride most rapidly. Wallace and Cushny (29), studying the intestinal absorption of saline catharties, found that the salts arrange themselves in order of the speed of absorption as follows:—chloride, bromide, iodide > sulfate > phosphate, tartrate, citrate.

The effect of calcium. Calcium was studied in these experiments first because, as Loeb (30) and Osterhout (31) have pointed out, it exhibits an antagonism toward sodium and secondly because of its alleged action on the permeability of membranes. In experiments on the prevention of pleural exudates in dogs, Chiari and Januschke (32) reported that calcium injected subcutaneously inhibited the transudation after treatments which in control dogs caused a copious amount of fluid. The whole scheme of experimentation was rather severe in this investigation. The appearance of icterus after the injection of fluorescein was found by Rosenow (33) to be distinctly inhibited by calcium chloride.

Small amounts of concentrated sodium chloride injected intravenously in rabbits elicit a hyperglycemia which is prevented by adding calcium chloride to the salt solution (34). Slow injections of large volumes of sixth molecular sodium chloride into rabbits produce a glycosuria which is prevented by the addition of three-eighths molecular calcium chloride in the ratio 975 cc. NaCl to 25 cc. $CaCl_2$ (35). MacCallum (36) found that saline purgatives injected intravenously produced active peristalsis but that the action was inhibited by calcium chloride. Calcium antagonizes not only sodium but also magnesium (37) and potassium (38), (39), (40), (41).

In the present experiments calcium chloride was injected with sodium chloride in isotonic solution to ascertain whether or not the calcium would hinder the movement of fluid out of the capillaries. As will be seen from table 4, instead of inhibiting the return to normal relative blood volume, the presence of the calcium seemed, if anything, to hasten the outward passage of the injected fluid. Yanagawa (42) had reported that calcium chloride does not reduce the permeability of the capillaries under normal conditions.

In the second injection of experiment 31, 0.25 gram calcium chloride dissolved in isotonic sucrose solution was injected and the animal died with tetanic convulsions. The left ventricle was in strong contraction

and there was a clot in the right auricle. When a higher concentration of calcium chloride in sodium chloride solution was injected there were no untoward effects. This seems to indicate that the sodium exerted an antagonism not shown by the sucrose.

TABLE 4

Regulation of blood volume after injection of sodium chloride containing calcium chloride, relative blood volume

TIME INTERVAL	EXPERIMENT NUMBER		
	32 (First injection)	32 (Second injection)	33 (First injection)
	250 cc. 0.9 per cent NaCl 6.4 cc. $\frac{2}{3}$M $CaCl_2$	250 cc. 0.9 per cent NaCl 12.8 cc. $\frac{2}{3}$M $CaCl_2$	250 cc. 0.85 per cent NaCl 20 cc. $\frac{2}{3}$M $CaCl_2$
minutes			
Normal	100	100	100
Immediately	123	141	134
5	108	113	110
10	102	105	100
15	100	105	100
20	100	100	100
25	100	100	100
30			
40			
50			
60			

The effect of colloidal silver. Colloidal silver is a suspensoid colloid while the blood proteins are emulsoid colloids. Spiro (43) has reported that the imbibition of water by gelatin is accelerated by the presence of colloidal iron oxide. The present experiments were carried out to determine the effect of the interaction of the two types of colloids upon the movement of water out of the circulation. The technic was the same as used before in the present experiments. The silver preparation used (Solargentum, Squibb)[2] is said to contain about 20 per cent silver It was dissolved in 0.97 per cent sodium chloride solution and in three cases the dosage was 50, 100 and 200 mgm. respectively dissolved in 100 cc. of the salt solution. It caused no depression of the freezing point and gave a golden brown solution which did not interfere with the hemoglobin determinations.

[2] This preparation was kindly furnished through Dr. I. F. Harris by E. R. Squibb & Sons.

Table 5 shows the results of injecting colloidal silver solutions. When the amount injected was 50 or 200 mgm. there was no appreciable effect on the return to normal of the relative blood volume. When 100 mgm. were injected the relative blood volume remained above normal. This injection was, however, the second dose of colloidal silver that rabbit had received. It appears that there may be a cumulative effect of the colloidal silver solution. There was glycosuria in all cases after the injection of colloidal silver. This point will be investigated further.

TABLE 5

Regulation of blood volume after injection of colloidal silver solution (Solargentum Squibb); relative blood volume

TIME INTERVAL	EXPERIMENT NUMBER		
	44 (First injection)	44 (Second injection)	45
	100 cc. 0.97 per cent NaCl 50 mgm. silver	100 cc. 0.97 per cent NaCl 100 mgm. silver	100 cc. 0.97 per cent NaCl 200 mgm. silver
minutes			
Normal	100	100	100
Immediately	143	132	142
5	122	118	129
10	106	113	106
15	104	113	106
20	106		101
25	104	111	101
30	104	111	101
40	104	111	100
50			
60			

The effect of acacia. Since varying accounts of the value of acacia in shock have been reported, it seemed of interest to try its effect in normal animals under the conditions of our experiments. In circulatory shock saline solution or Ringer's solution given intravenously leaves the circulation so rapidly that it helps little. Bayliss (44), (45), (46) introduced the use of gum acacia and has been its foremost champion. The value of acacia lies in the fact that it possesses not only viscosity but also a small osmotic pressure thus simulating the plasma proteins. He recommends the intravenous use of 7 per cent acacia in 0.9 per cent sodium chloride solution in the treatment of shock on the theory that if the blood pressure and aeration are kept up acidosis need not be feared.

A study of the blood volume regulation in normal animals after the injection of acacia was made in the present investigation according to the technic already described, a solution of 7 per cent acacia in 0.9 per cent sodium chloride ($\Delta = 0.59$) solution being used. The Δ due to the acacia alone was 0.07.

Table 6 shows that considerable fluid passed out of the circulation during the injection. The acacia maintained the augmented blood volume in a way not observed with any other substance used in these experiments. It held the relative blood volume one-third above normal for more than an hour.

TABLE 6

Regulation of blood volume after injection of acacia-sodium chloride solution; relative blood volume

TIME INTERVAL	EXPERIMENT NUMBER		
	33 (Second injection)	52	53
minutes			
Normal	100	100	100
Immediately	153	141	144
5		141	137
10	146	141	137
15	146	141	137
20		141	137
25	136	141	137
30	134	141	134
40	133	141	131
50	133	135	131
60	133	129	131

Moore (47) attributes the value of acacia to the restoration of the hydrophilous colloids to the blood. That it exerts a small persistent osmotic pressure and thus maintains the blood volume has been asserted by Gasser, Erlanger and Meek (48). Kruse (49), however, has suggested that the value of acacia in maintaining blood volume may be due to its adsorption on the capillary walls, whereby the exit of the fluid from the vessels is impeded.

The effect of acid. The effect of acid on the swelling of colloids and on the permeability of membranes has been widely studied. Spiro (43) reported that $\frac{M}{500}$ hydrochloric acid caused gelatin discs to swell more than did water, while Chiari (50) showed that increased swelling was obtained when carbon dioxide was present in conductivity water. Fischer (51) demonstrated the swelling of muscle in acid. Osterhout (52) studying diffusion, found that acid first decreased the permeability of the cell membrane but later increased it. Harvey (53) showed that all acids excepting benzoic and salicylic encounter resistance to diffusion at the surface of the living cell.

It might appear a priori that injection of acid would increase the water-holding capacity of the plasma colloids or would decrease the permeability of the capillary membrane to such an extent that the passage of fluid out of the circulation after injection would be inhibited. On the other hand, the body has such remarkable ability to take care of increased acid and to maintain its constant reaction (45) that it would appear problematical whether or not one could introduce enough acid to change the physical chemical equilibria without killing the animal.

TABLE 7

Regulation of blood volume after injection of hydrochloric acid-sodium chloride solutions; relative blood volume

TIME INTERVAL	EXPERIMENT NUMBER			
	34	38 (Second injection)	54	56
	$\frac{M}{20}$ HCl in 0.7 per cent NaCl	$\frac{M}{15}$ HCl in 0.6 per cent NaCl	$\frac{M}{15}$ HCl in 0.6 per cent NaCl	$\frac{M}{15}$ HCl in 0.6 per cent NaCl
minutes				
Normal	100	100	100	100
Immediately	117	124	121	138
5	111	111	108	115
10	108	108		109
15	104	105	104	103
20	104	102	108	103
25	102		104	101
30	101	108	104	103
40		105	102	101
50			104	101
60			104	103

To test this point with reference to changes within the blood vessels, $\frac{M}{20}$ and $\frac{M}{15}$ hydrochloric acid made isotonic with 0.7 per cent and 0.6 per cent sodium chloride respectively were injected as in experiments already recorded. $\frac{M}{15}$ acid seemed to be the limit of tolerance under the conditions of the experiment. Several of the rabbits died during the injection and on autopsy showed pulmonary edema. During infusion there were in every case dyspnea and spasmodic contractions of the voluntary muscles.

From table 7 it will be seen that in the concentrations used hydrochloric acid failed to decrease the rate of passage of fluid out of the blood vessels. The relative blood volume returned to normal as rapidly when acid-sodium chloride solutions were used as with the sodium chloride alone.

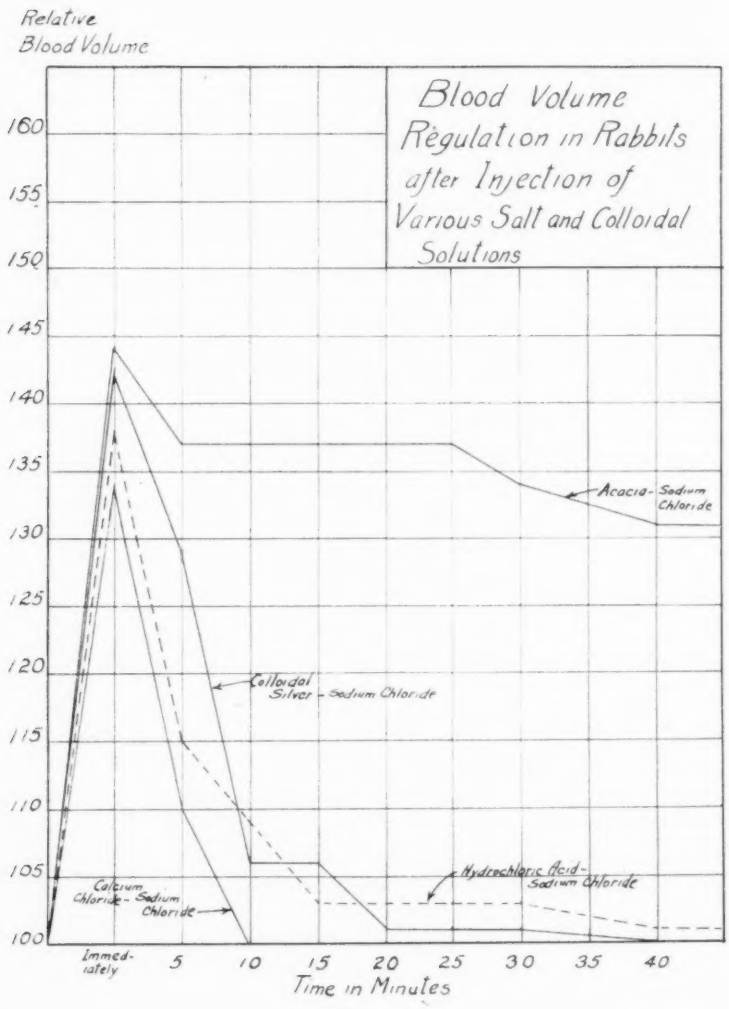
Relative
Blood Volume
Blood Volume Regulation in Rabbits after Injection of Various Salt and Colloidal Solutions
160
155
150
145
140
135
130
125
120
115
110
105
100
Acacia - Sodium Chloride
Colloidal Silver - Sodium Chloride
Hydrochloric Acid - Sodium Chloride
Calcium Chloride - Sodium Chloride
Immed-iately
5
10
15
20
25
30
35
40
Time in Minutes

Chart 3

The effect of sucrose. To determine the effect of a crystalline non-electrolyte on the removal of fluid added to the blood 10.9 per cent sucrose solution ($\Delta = 0.59$) was injected in one experiment. From table 8 it will be seen that the relative blood volume returned to normal as rapidly as with saline solution.

TABLE 8

Regulation of blood volume after injection of sucrose solution; relative blood volume

TIME INTERVAL	EXPERIMENT NUMBER 31
minutes	
Normal	100
Immediately	158
5	146
10	119
15	109
20	104
25	101
30	100
40	100
50	
60	

Fate of the injected fluid. From the preceding experiments it is evident that with the exception of the citrate, tartrate, sulfate and acacia none of the substances injected decreased the rate of loss of fluid from the circulation as judged by the variation in blood volume. In the majority of cases a volume of fluid equal to the blood volume diffused out of the circulation in less than one half-hour. In speculating upon, and, if possible, determining the fate of this fluid, attention is directed to the possible paths of elimination of this excess of volume. It may be excreted through the kidneys, it may accumulate as edema fluid, it may form serous exudates, it may pass into the tissue spaces or it may be excreted into the gastro-intestinal tract.

Haldane and Priestley (54) have shown in a striking manner that copious water drinking results in a large urine output. The effect of water drinking upon the circulation must be analogous to that produced by the injection of isotonic solutions in our experiments. Magnus (55), (56) concluded that the cause of the diuretic action of salts lies in the composition of the blood and not in the increased capillary pressure. He found that sulfate solutions of the same osmotic pressure as chloride solutions produced greater diuresis.

TABLE 9

Urine volume as percentage of injected solution

SOLUTION	EXPERIMENT NUMBER	PER CENT	AVERAGE
Sodium sulfate	2	55	
	7	64	
	20	51	
	27	110	70
Sodium nitrate	4	28	
	5	66	
	17	63	
	28	22	
	30	37	43
Colloidal silver	44	16	
	45	29	23
Sodium citrate	36	28	
	37	21	
	38	14	21
Sodium sulfocyanate	3	27	
	14	17	
	23	13	19
Sodium acetate	15	15	
	22	23	19
Calcium chloride-sodium chloride	32	14	
	33	20	17
Sodium tartrate	46	26	
	48	8	
	48	7	13
Hydrochloric acid-sodium chloride	34	10	
	54	16	
	55	8	11
Sodium chloride	9	6	
	11	12	
	18	8	9
Sodium bromide	10	8	
	12	8	
	13	12	9

Table 9 shows the average figures for urine volumes excreted during the various experiments and calculated as percentages of the injected volume. Sulfate and nitrate induced the largest secretion of urine observed with any of the salts or substances investigated. This corresponds with the statements in the literature on the comparative diuretic effect of these salts. However, even in the sulfate experiments where the salt caused the greatest diuresis the urine volume did not account for the fluid which had left the circulation.

The nature of edema and the factors involved in its production have long been matters of discussion. Cohnheim and Lichtenheim (57) injected salt solution until they had given 46 per cent of its weight to a rabbit and 64 per cent to a dog yet they failed to observe subcutaneous edema. On infusing salt solution after arsenic poisoning, Magnus (58) produced edema although in normal animals plethora elicited no edema. It is evident from the literature that hydremic plethora will not produce the edema which is characteristic of nephritis. In the present experiments no patent edema was observed in any of the animals.

The pleural and peritoneal cavities offer considerable free space for the accumulation of pathological fluids. After injection of saline solution Cohnheim and Lichtenheim (57) found fluid in the peritoneal cavity but the pleural cavity was dry. Of the fluid injected into rabbits, Dastre and Loye (59) found 75 per cent in the tissue spaces and serous cavities. In the present experiments the pleural cavity was, as a rule, normal in appearance. The peritoneal cavity usually contained fluid but there was never more than 5 cc. of transudate which usually contained protein and the salt injected and would clot. It is certain that the fluid in the peritoneal cavity accounted for very little of that which diffused out of the blood vessels.

The indefinite area known as "tissue spaces" constitutes a reservoir of fluid in the animal body. After hemorrhage the volume of blood is brought back toward normal by the passage of lymph from the muscle and other tissues into the circulation. Hypertonic solutions draw part of the fluid which renders them isotonic from the tissue spaces. Engels (60) reported that 68 per cent of the fluid injected into a dog was contained in the muscles. In the present investigation experiments were carried out to determine whether or not the amount of water contained in the muscle was demonstrably increased. From table 10 it will be seen that the percentage of water did not vary after the injection. This was rather surprising after the statements made in the literature. All of the older experiments dealt with slow infusions extending over a

long period of time. Perhaps the manner in which we carried out the experiment altered the distribution of the fluid.

The excretory function of the small intestine has been given considerable attention in the literature. In the course of their experiments on hydremic plethora, Cohnheim and Lichtenheim (57) observed that the stomach and intestine became swollen. Albu (61) reported that after injection the intestinal contents became a thin paste. J. B. MacCallum (62) injected large volumes of normal saline solution intravenously; and from a cannula tied in the intestine he obtained fluid which amounted to 15 per cent, 9 per cent and 10 per cent of the injected volume in three different experiments.

TABLE 10

The water content of muscles before and after injection of isotonic salt solutions

	GASTROCNEMIUS	TIBIALIS ANTICUS
	per cent	*per cent*
Experiment 41		
Before injection	78.0	77.8
After injection	77.5	77.5
Experiment 42		
Before injection	76.0	76.5
After injection	76.2	76.2
Experiment 43		
Before injection	79.5	79.5
After injection	79.3	79.4

In the present experiments abnormal distention was repeatedly noticed which, on autopsy, was seen to be due to the injection of the stomach, small intestine and cecum. The mass of material in these organs was a thin paste and on section fluid streamed from the stomach. These observations indicate that, under the conditions of our experiments, some fluid was excreted into the gastro-intestinal tract.

From the above discussion it appears that the part of the injected fluid which diffused out of the blood stream in these experiments can be accounted for in the urine, in the exudations into the serous cavities and in the excretion into the intestinal tract. In these experiments there was, however, no trace of edema nor was there detectable increase in the water content of the skeletal muscles.

SUMMARY

When isotonic solutions of the acetate, nitrate, sulfocyanate, bromide, chloride, tartrate, sulfate or citrate of sodium are injected intravenously at such a rate that a volume equal to the estimated blood volume is introduced in two minutes, the rate at which the added fluid escapes from the circulation, as measured by the relative blood volume at successive subsequent intervals, is decreased to a slight extent by the sulfate, tartrate and citrate.

When calcium chloride, hydrochloric acid or colloidal silver was dissolved in sodium chloride solution and was injected intravenously there was no alteration of the rate of return to normal blood volume.

When acacia-sodium chloride solution was used there was a marked and long sustained increase in the relative blood volume.

Sucrose in isotonic solution did not delay the passage of fluid from the blood vessels.

The fluid which leaves the circulation in the restoration of blood volume after the injection could not be accounted for by the passage into the muscles or by edema fluid. The volume of urine, exudate into the serous cavities and the excretion into the intestine and stomach probably are concerned in the disposal of the fluid leaving the circulation.

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